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PAIN IN MUSCULAR ISCHEMIA

ITS RELATION TO ANGINAL PAIN

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The term angina pectoris is usually and properly used to indicate the disease as it was described by Heberden and his contemporaries. It is not with the general pathology of this disease that I propose to deal, but rather with one problem related to it, namely, the origin of the pain that characterizes the attacks that render this malady so distressing. There is perhaps no symptom familiar to clinicians that has given rise to more speculation than that of anginal pain. The theories that have been put forward to explain it are too numerous to pass even in review; such a review will not be attempted, but the problem will be approached from a special angle that is relevant to the new observations to be described.

The idea that angina pectoris can arise out of a morbid change in the coronary vessels came, as Parry [1] has said, from Edward Jenner. His idea was being fostered a hundred and fifty years ago in the small Gloucestershire Medical Society of which Jenner, Parry and Paytherus were members. From his letter to Heberden [2] it may be inferred, perhaps, that Jenner believed these vessels to be unable to perform their functions, and that this failure of function is responsible for the painful spasms. It is, however, in the writings of the distinguished Welsh physician Parry that the baneful effects of cardiac ischemia are first expressed with clarity. His words constitute so important a step in the history of this subject that they are here quoted:

> The rigidity of the coronary arteries may act, proportionably to the extent of the ossification, as a mechanical impediment to the free motion of the heart; and though a quantity of blood may circulate through these arteries, sufficient to nourish the heart, as appears, in some instances, from the size and firmness of that organ,

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1. Parry, C. H.: An Inquiry into the Symptoms and Causes of the Syncope Anginosa, Commonly Called Angina Pectoris, London, Cadell & Davis, 1799, pp. 3, 113 and 114.

2. Baron, J.: Life of Edward Jenner, London, H. Colburn, 1827, p. 39.

yet there may probably be less than what is requisite for ready and vigorous action. Hence, though a heart so diseased may be fit for the purposes of common circulation, during a state of bodily and mental tranquillity, and of health otherwise good, yet when any unusual exertion is required, its powers may fail, under the new and extraordinary demand. Accordingly we find, that paroxysms of the Syncope Anginosa are readily excited by those passions, the tendency of which is to stimulate the heart to excessive contraction.[1]

This clear and important statement is one of Parry's chief contributions to the knowledge of angina pectoris. It is not, however, the statement for which he is usually remembered. Parry was convinced of the essential part played by syncope in the disease, and to him the syncope of angina pectoris was equivalent to failure in the strength of the heart beat and led to cessation of its motion. Parry overemphasized the frequence and therefore the importance of syncope. Although in his recapitulation he said that angina pectoris "is a case of syncope, preceded by a notable anxiety or pain in the region of the heart," thus acknowledging the pain as of early occurrence in the attack, subsequently he described pain extending from the breast to the left arm as an "accidental symptom" and as one occurring in other states. Thus, while Parry brilliantly conceives the heart to be crippled in its reserve by the incapacity of its nutrient vessels and thus explains the spasmodic nature of the symptoms, yet he keeps pain in the background and brings weakness of the heart beat into the foreground. The impressive writings of Parry were well known to Burns,[3] and the leading ideas in the following extract and in other relevant passages of Burns' book coincide with and are clearly derived from those of Parry. Thus Burns said:

In health, when we excite the muscular system to more energetic action than usual, we increase the circulation in every part, so that to support this increased action, the heart and every other part has its power augmented. If, however, we call into vigorous action, a limb, round which, we have with a moderate degree of tightness applied a ligature, we find that then the member can only support its action for a very short time; for now its supply of energy and its expenditure, do not balance each other; consequently, it soon, from a deficiency of nervous influence and arterial blood, fails and sinks into a state of quiescence. A heart, the coronary vessels of which are cartilaginous or ossified, is in nearly a similar condition; it can, like the limb, be girt with a moderately tight ligature, discharge its functions so long as its action is moderate and equal.

Burns here compares the heart and the limb to which the blood flow is impeded, but in doing so he followed Parry's lead and wrote, not of pain, but of an organ failing and sinking into a state of quiescence, as did Kreysig[4] a few years afterward and Benjamin Brodie,[5] forty-

3. Burns, A.: Observations on Some of the Most Frequent and Important Diseases of the Heart, etc., Edinburgh, Bryce & Company, 1809, p. 138.

4. Kreysig: Die Krankheiten des Herzens, 1816, pt. 2, p. 512.

5. Brodie, B. C.: Lectures Illustrative of Various Subjects in Pathology and Surgery, London, Longman [and others], 1846, p. 360.

seven years after Parry,[6] in making similar comparisons. As will be seen a little later, when a voluntary muscle is deprived of circulation and is vigorously worked, pain and not weakness brings its movements to an end. If one holds Parry's view that, in anginal attacks, weakness of beat and syncope are common manifestations, then Burns' analogy would possess more point, and it would be more important if one were considering only prolonged anginal attacks threatening to deprive or actually depriving the subject of life. Burns' analogy has not the same historical significance in relation to the production of pain, which is here under particular consideration. It is not until one considers the work of Potain[7] (1866), who had behind him the full accounts of his countrymen on "intermittent claudication," that one finds pain arising from heart and from the limb clearly compared. Thus, the important idea of cardiac pain arising out of an ischemic condition[8] of the working organ has grown slowly but steadily toward clarity; it has not failed to find notable support[9] (as by Balfour,[10] Huchard[11] and Mackenzie[12]).

If one is to grasp fully the possible significances of comparing pain arising in a working limb and in the heart, each inadequately supplied by blood, and if one is to ascertain if the comparison leads one ultimately beyond a simple analogy to find a fundamental pain factor common to the two instances, it will be necessary to analyze the mechanism of such pain more closely and to obtain clearer ideas of the factors influencing it than has been done hitherto. The idea is too important to be left at the stage of hypothesis.

I shall begin, therefore, by considering at some length how in the case of the limb such pain arises.

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6. Brodie, however, was the first to draw the analogy between intermittent claudication and angina.

7. Potain, in Dictionnaire encyclopédique des sciences médicales, Paris, V. Masson et fils, 1866, vol. 4, p. 346.

8. While it will be agreed that pain is an almost constant and a chief feature of the anginal seizure, the idea that ischemia of the heart is responsible for no more than pain is one that could scarcely be allowed. The text emphasizes the symptom pain because pain is the symptom the mechanism of which is under consideration.

9. A clear account has recently been published by C. S. Keefer and W. H. Resnik (Angina Pectoris: A Syndrome Caused by Anoxemia of the Myocardium, Arch. Int. Med. **41**:769 [June] 1928).

10. Balfour, G. W.: The Senile Heart, Its Symptoms, Sequelae, and Treatment, London, A. & C. Black, 1896.

11. Huchard, Henri: Traité clinique des maladies du coeur et des vaisseaux, Paris, O. Doin, 1899, vol. 2, p. 151.

12. Mackenzie, James: Angina Pectoris, London, Oxford University Press, 1923, p. 36.

THE ORIGIN OF PAIN IN ISCHEMIA OF THE LIMB

Two main views have been held in attempting to account for the pain in ischemia of the working limb. One of these, which is still chiefly favored on the European continent, is that pain comes from arterial spasm.[13] This view may be dealt with briefly and excluded, since Pickering, Rothschild and I [14] have found it easy to show that when the muscles of the forearm are exercised while deprived of blood supply, the vessels to the corresponding part of the limb actually lose their tone. If the circulation to an arm is arrested and the muscles of the forearm are exercised vigorously until pain is intense, plethysmographic records demontrate that, immediately after the circulation is released and exercise is stopped and while pain still continues, the forearm is flooded with blood owing to the vessels being at the time widely open.

The second view, which has been more favored in England, is that the pain is of muscular origin. Charcot [15] was first responsible for this view, though he had the idea that the muscle enters on a condition of cramp, which he likened to "cadaveric rigidity," an idea that, as will be seen later, is untenable. There has been, in fact, little or no observational work that throws light on the mechanism of the pain under consideration, and, recognizing this, Dr. Pickering, Dr. Rothschild and I undertook a series of new observations,[14] which will now be discussed.[16]

The exercise used has been a simple gripping movement. The contractions are almost maximal, at the rate of one a second. The circulation is arrested during the exercise by means of a sphygmomanometer cuff, which is distended to a high pressure on the upper arm. In an observation of this kind, pain begins from about twenty-five to forty-five seconds (or the same number of contractions) after the beginning of exercise; it develops progressively, increasing in intensity and rendering a continuation of exercise, still vigorously pursued, very disagreeable or intolerable at about the seventieth second. It is to be emphasized that if the exercise is done under suitably controlled conditions, the time

13. The corresponding idea has been held also in the case of the heart; namely, that anginal pain arises directly out of spasm of the coronary arteries in cardiac ischemia (see Gallavardin, L.: Les angines de poitrine, Paris, Masson & Cie, 1925, p. 126).

14. Lewis, Thomas; Pickering, G. W., and Rothschild, P.: Heart **15**:359, 1931.

15. Charcot: Progrès méd. **6**:99, 1887.

16. The fact that pain occurs in muscles exercised in the absence of a blood supply has long been known. It was an observation frequently referred to by Mackenzie. Zak studied the phenomenon (in 1921), but, as we think, misinterpreted it. In our original paper we overlooked MacWilliam and Webster's article (Brit. M. J. **1**:51, 1923); this paper deals, in the main, with the relation of pain and fatigue, but here and there covers ground similar to our own.

at which pain develops to the point that for convenience is termed intolerable can be estimated by the subject within narrow limits of error, and the time proves to be remarkably constant in repeated tests by one subject and in different tests by different subjects. The pain has a peculiar and characteristic aching quality. Although it may be described as rather diffuse, yet it is felt maximally in the region of the muscles most exercised. Thus the situation in which it appears can readily be altered by throwing the burden of exercise mainly on this or on that group of muscles. An important point is that the pain is continuous; it does not increase appreciably with the contraction of the muscle, and it does not subside with the relaxation. It is a smooth pain, gradually but constantly growing in intensity while exercise lasts. Thus, it is shown to be independent of the tension developed during the contraction of the muscle.

The pain is not the result of cramp, for in none of our observations has there been any sign of tonic contraction, relaxation being complete between each movement. We are not prepared to say that cramp never occurs as an accompaniment, but it is quite clear that cramp is unessential to the production of the muscular pain described.[17] Tenderness is developed in the region where pain has been felt, namely, in the group of muscles most exercised, and may last for a long time after the pain has disappeared.

We have shown that the pain is not the direct result of a lack of oxygen. Of these observations, the most conclusive is perhaps the following: The forearm, deprived of its blood supply, is exercised in the usual way, and the exercise is continued until pain begins; this time is noted. After a period of rest, the exercise is repeated in exactly the same way, but it is stopped a few seconds before pain is anticipated. Although the arterial occlusion is prolonged for a further period of five minutes, no pain develops. Now if pain is to be attributed to lack of oxygen, it is clear that at the instant exercise ends the amount of oxygen in the tissues must have declined almost to the necessary level for the production of pain. Yet, although during the immediately succeeding period oxygen will still be used up rapidly owing to the oxygen debt that has been established in the muscle, no pain develops. Thus it is impossible to accept the view that in this experiment pain fails to develop simply because the oxygen deficiency is not carried far enough. The relationship is not to the lack of oxygen but to the muscular exercise, and the time at which pain appears is governed by the amount of exercise taken. If the rhythm of muscular contraction remains con-

17. P. M. Latham (Lectures on Subjects Connected with Clinical Medicine, etc., London, Longman [and others], 1846, vol. 2, chap. 37) believed that cramp of the heart muscle causes angina, but this idea now finds little or no support.

stant, then the pain begins earliest in those exercises in which the tension developed at each contraction is greatest. On the other hand, if the tension developed is kept constant, then the time at which pain develops is controlled by the rhythm of the contractions; the faster the rhythm, the quicker the pain will come. Usually the pain comes after a given number of contractions developing a given tension, whether the rhythm of contraction is slow or fast. From these experiments and from supporting evidence presently to be given, it is concluded that when muscles deprived of blood supply are exercised, the pain that develops is due to a stimulus arising directly or indirectly out of the contraction process.

A most important clue to the nature of the pain mechanism is provided by the following very simple observations. The circulation to the arm is arrested, and exercise is undertaken until pain develops to a given degree. If the exercise is now stopped but the circulatory arrest is continued, the pain does not subside but continues. It continues at the intensity that has been developed by the time exercise ends, and it is maintained at this intensity so long as the circulation remains arrested. But if the circulation is released at any time subsequently, the pain disappears completely within from two to four seconds. This observation has led us to the view that the pain is determined by some chemical or physicochemical agency within the mass of muscle, for the factor underlying pain continues unchanged until the circulation is restored, when it is promptly removed.

It will perhaps facilitate description if I anticipate and state that we believe the stimulus causing pain to have its seat in the tissue spaces; the reasons for this belief will appear later. When a muscle contracts, changes such as the release of metabolites occur within its fibers; one obvious possibility is that these metabolites diffuse out and comprise the stimulating agent in the spaces; however, we wish to avoid committing ourselves to a conclusion in this form, since it is also possible that changes within the fiber may induce secondary and distinct changes within the spaces. So it becomes necessary to keep relevant changes within and without the fiber as distinct ideas; we do so by naming the latter the pain factor or "factor P," because it constitutes the immediate stimulus to pain.

When a muscle is exercised under ischemic conditions, factor P, once formed, remains unchanged. Moreover, it is cumulative, increasing in amount with each muscular contraction; it rises first to a level adequate to bring pain and then to higher levels, associated with increasing pain. Because it is stable during circulatory arrest, it maintains the pain between muscular contractions and after exercise has ceased.

Consider next the interpretation of what happens when the circulation to the muscles is released. In the usual exercise the time taken

for pain to develop from its beginning to its intolerable point is about thirty-five seconds. The amount of factor P responsible for this development of pain is dispersed in from two to four seconds of the restoration of the blood flow to the muscles. This rapid recovery is, in our view, a recovery confined to the tissue spaces. Recovery of the underlying process in the fiber itself takes much longer. If, a little time after release, the circulation is rearrested and the exercise is repeated, the time taken for pain to reappear is shorter than in the original exercise, and the amount of shortening is related to the shortness of the period of release intervening. Actually, it is found that this period of release between two exercises must extend to ten minutes if the times for the development of pain in the first and second exercise are to be made to correspond. This interval of ten minutes is the approximate time taken for the recovery of the muscle fiber, so far as those processes ultimately underlying the production of pain are concerned.

If the exercise is begun and the circulation is arrested some time later, the exercise being continued, the preliminary period of exercise with free circulation is unaccompanied by pain, but it shortens the time taken for pain to develop in the subsequent period of exercise under ischemic conditions. Observations of this kind indicate that during muscular exercise with the circulation free the change within the muscle fibers that ultimately underlies pain occurs, but pain does not develop, since factor P cannot accumulate in the tissue spaces while these are under the influence of a free stream of blood. Confirmation of this view is found in experiments of the following type. If the exercise is undertaken with the circulation free for a period of two minutes and if the blood flow is then arrested and the exercise stopped simultaneously, there is no pain, but pain develops after a latent period of about from twenty to thirty seconds and may become severe within one minute. In this instance, the processes underlying the production of pain occur during the exercise, but factor P cannot rise to a corresponding level in the tissue spaces until some time after the circulation ceases.

When pain has developed in the usual exercise under ischemic conditions and has been abolished by release of the vessels, the pain returns after a latent period if the blood supply is rearrested. The latent period is shorter and the intensity of the pain developed is greater if the period of release is short. In this instance, factor P is reduced or abolished in the tissue spaces during release and accumulates on rearrest; the level to which it reaccumulates naturally depends on the amount by which the muscle fiber recovers during the release.

These are the main evidences for the conclusion already formulated that factor P acts in the tissue spaces, but is dependent on processes occurring within the muscle fiber as a result of its contraction. When the blood flow is arrested, the process in the muscle and the accumulation

of factor P in the tissue spaces occur *pari passu;* with the circulation free, the same process occurs in the muscle fiber, but the condition of the tissue spaces is kept relatively unchanged.

The observations here briefly recorded [18] are relevant to the main thesis of this article. They afford, for the first time, evidence for the belief that in ischemia pain arises from a working muscle, and that the stimulus is derived from physiologic processes in the muscle fiber. Further, if the characteristics of anginal pain and the various circumstances in which it arises and is influenced are closely considered in the light of these observations on somatic muscle, much that was previously obscure becomes plain to the understanding.

ANGINAL PAIN

Anginal pain is believed to result from relative ischemia of the muscle. This view does not ascribe it to a simple mechanical cause, but recognizes that it may be provoked in distinct ways. Thus, it may be provoked if the blood supply to the muscle becomes diminished, while the work done remains constant, or it may be provoked if the blood supply fails to increase adequately to the needs of a muscle that is called on to increase its work.

Coronary Occlusion; Diminished Blood Supply.—One of the most powerful arguments for the theory that anginal pain is caused by muscular ischemia is the symptomatology of acute coronary occlusion. It is established beyond any reasonable doubt by clinical observation that pain is produced by occlusion of the coronary vessels, and that this pain is indistinguishable from that of angina pectoris in situation, radiation and character. If, as would appear, the pain of coronary occlusion is more frequently epigastric than is the other, the fact is still unexplained; however, coronary pain often starts in other places, in the region of the upper part of the sternum, over the precordium or even in the left arm, as does the pain of angina pectoris. Starting usually in the chest, it radiates to the left arm, both arms, the neck, jaw, abdomen or scapula, as does spasmodic anginal pain. The unfortunate sufferers in the two groups use a precisely similar series of descriptive terms in trying to make known the character of the pain. In both groups a sense of weight or constriction as an integral part or accompaniment is referred to frequently, as is also alarm that death is coming. Those who are interested may profitably study the fine series of case notes published with autopsies by Levine.[19] In clinical cases, the

18. A full statement of the results will be found in a contemporary publication (footnote 14).

19. Levine, S. A.: Coronary Thrombosis: Its Various Clinical Features, Baltimore, Williams & Wilkins Company, 1929.

occlusion is usually by thrombosis (sometimes by embolism), forms of obstruction that are open to the very least objection as uncomplicated instances of arrested blood flow. The patients afford a much safer source of information regarding the symptom pain than do experiments on animals in which there is deliberate interference with the coronary vessels. Pearcy, Priest and Van Allen [20] and Sutton and Lueth [21] repeatedly observed evidences of pain, referred seemingly to the left foreleg, after ligature of a coronary vessel or its branch in dogs. These observations corroborate what has been established clinically. The chief value of Sutton and Lueth's work lies in their failure to awaken painful impulses from the heart in any other way than that of interrupting the coronary stream.

Since severe pain is known to occur when the coronary artery is occluded, whether by ligature or by internal clot, it is difficult to refrain from concluding that it originates from the heart, as it does from the limb, through muscular ischemia, and since in the latter case it is concluded that the underlying process occurs in the muscle fiber and is due to muscular contraction, the stimulus acting actually in the tissue space, it may be assumed that similar processes underlie the pain of coronary occlusion. All our evidence is quite compatible with this view. The pain of coronary occlusion is continuous; it does not come and go with the heart beat. It begins as a slight pain and grows steadily, and often rapidly, in intensity. In these features it is like the pain derived from somatic muscle in similar circumstances. In its continuity this pain differs from that produced by overloaded involuntary muscle, as, for example, the bowel, each separate contraction of which induces a simultaneous spasm of colic. Our theory explains this continuity. It is not tension developed in the heart that brings pain, but a chemical or physicochemical stimulation of its afferent nerves. This stimulus will begin to form in the tissue spaces from the instant of occlusion, and the pain will come the sooner in the case of the heart because the muscle is in continuous work at the moment of arrest. Sutton and Lueth called attention to the pain coming immediately or almost immediately, a point of much interest; if the circulation is arrested to somatic muscle already working continuously, the pain may begin within eight seconds, when the rhythm is sixty contractions a minute; it occurs more quickly if the rhythm of contraction is quickened. The almost instant appearance of pain in coronary occlusion is not inconsistent with the view put forward. The prompt relief when the ligature is released is entirely consistent with this view.

20. Pearcy, J. F.: Priest, W. S., and Van Allen, C. M.: Am. Heart J. **4**:390, 1929.

21. Sutton, D. C., and Lueth, H. C.: Pain, Arch. Int. Med. **45**:827 (June) 1930.

In thrombotic occlusion the pain lasts for hours or even days, and in this it differs chiefly from the pain in attacks of angina pectoris. According to our theory, it must be long lasting. When a coronary vessel is plugged, a corresponding area of muscle loses its blood supply; if in an experiment one watches the cardiac wall, the affected area becomes darker; gradually it loses its power to contract and balloons outward at each systole of the ventricle. The loss of function happens over a period of fifteen minutes or more. Long before this degree of weakness develops, according to experiences with somatic muscle, factor P, the pain stimulus, will be accumulated sufficiently to give intense pain. Once accumulated it will continue there, whether the muscle beats or not, and will give rise to pain as long as it can affect living sensory nerve endings. Sooner or later the nerves in the fully affected region will become paralyzed, and painful impulses will cease to ascend these; however, it is obvious that the nerves on the margins of the bloodless zone may be affected for much longer times.

Long-lasting pain is explained without difficulty. The duration will vary according to circumstance; one factor governing variation will be the rate at which the affected nerves die. Another factor that will influence the pain will be restoration of the blood flow, for the whole area of muscle that becomes ballooned does not necessarily die; a part or even the whole is often found in experiment to be restored to action by a return of blood through anastomotic channels. It suffices for the moment to note that conspicuous variations in the duration of pain, and even of its intensity from hour to hour, are possible theoretically; they are actually experienced.

Spasmodic Anginal Pain; Blood Supply Inadequate for Work Done.—No theory explaining the pain can stand that will not account for its appearance in attacks, and in angina pectoris the relation of such attacks to exercise and to emotion has been recognized from the earliest days of its description. It was indeed the spasmodic nature of the malady that led Parry to state his theory of relative ischemia, for this theory afforded a ready explanation. It was this spasmodic nature that led to the comparison with intermittent claudication, for in both instances it is to be supposed that the vessels can supply enough blood during rest but not during work.

It is instructive at this point to contrast two views. The belief [22] that attacks of anginal pain can result from increased strain on a structure such as the aorta is distinctly equivalent to the belief that it is the result of raised pressure, for that alone can throw the walls of the corresponding cavity temporarily into increased tension. Under

22. Allbutt, T. C.: Diseases of the Arteries, Including Angina Pectoris, New York, The Macmillan Company, 1915.

the theory of ischemia and as exemplified by our investigations of somatic muscle, if the blood supply remains constant, the attacks of pain will result from the increased expenditure of energy; this expenditure will increase when the tension (blood pressure) is raised; it will also increase if the rate of beating is increased, the tension remaining constant. In this connection, it is most interesting to note that cases have been recorded in which characteristic anginal pain has occurred constantly with paroxysms of tachycardia, for such paroxysms, while failing to raise blood pressure, greatly increase the energy expended by the cardiac muscle. The hypothesis of strain cannot account for pain in these circumstances; that of relative ischemia does.

To the belief that angina is the result of mechanical strain, the similar appearance of the anginal attack at given levels of blood pressure is essential; to the theory of muscular ischemia, it is unessential. It will be evident how important is full knowledge of the changes of blood pressure and pulse rate in anginal seizures; yet, while much has been written on what is assumed to occur, little observational work has been undertaken to ascertain whether the facts do or do not fit with theory. It is easy to assume, because the blood pressure and pulse rate are known to be raised by exercise under normal conditions, that exercise always raises them when it provokes anginal pain. I do not doubt that such changes will be found, will prove adequate and will thus come more fully to justify explanations of anginal pain that are based on them, but the assumption remains, and is dangerous, until the pressures and rates have been read. When, as is so common in patients with angina, walking rather briskly on level ground induces the early tightness across the front of the chest, is one right in believing that there is, in fact, a rise in the expenditure of energy of the heart adequate to produce pain, and that an equal rise, however provoked, will always produce pain? The answer will come from appropriate observation and experiment.

There is a feature manifested by anginal pain provoked by effort that is not unusual, which was first described in that historic and remarkable letter of an unknown gentleman to Heberden.[23] He wrote: "I have frequently, when in company, borne the pain, and continued my pace without indulging it; at which times it has lasted from 5 to perhaps 10 minutes, and then gone off." That is a phenomenon difficult to explain on a simple basis of pressures and rates, but is easy to understand if it is believed that the coronary circulation accommodates itself gradually to the condition of exercise. It is, of course, premature to speculate in this way; it would be more satisfactory if one waited.

23. Medical Transactions published by the College of Physicians in London, 1875, vol. 3, p. 3.

What actually happens to the pressure over the time pain is disappearing under exercise can be, but has not yet been, ascertained. If I express the believe that no appreciable change will be found in it, I am anticipating what should first be demonstrated one way or another. My purpose here is not to use assumptions as arguments, but merely to point to obvious gaps in the knowledge in relation to anginal pain produced by effort.

I shall now consider another type of angina, namely, a type that occurs in patients at rest, and here I have definite and relevant information to offer.

From personal observations and by searching past records, I have been able to bring together a group of cases of a given type. A general description of this type, already exemplified elsewhere,[24] will suffice.

The Syndrome.—The syndrome may be described on the basis of my own series of cases and the single cases of Brunton,[25] Hunter,[26] Powell [27] and Mackenzie,[28] all of which are grouped together. Repeated and close observations have been made on patients of this type during anginal attacks, the pulse rate and blood pressure being studied in particular. The cases almost always occur in male patients who have free aortic regurgitation, though I have met with the characteristic syndrome in one middle-aged man in whom no signs of aortic valvular disease could be found. The patients have grave cases of heart disease, are often bedridden and at the most are capable of only slight exercise. They may live precariously for years; sudden death among them is common.

The pain has the usual, though variable, characteristics of grave angina pectoris. It is located at first in the front of the chest centrally, over the upper part of the sternum, or, less commonly, near the left nipple. It is a continuous, boring or gripping pain, acquiring in the major attacks an unbearable quality; it spreads characteristically into the arms, especially into the left. It may or may not be associated with a feeling of intense anxiety. In the earlier stages of the malady, the attacks of pain, which last from a few minutes to half an hour or more, may be provoked by exercise, in which case they bring the patient to a state of immobility. In later stages, when little exercise is possible, they occur while the subject is at rest, and seem particularly prone to happen in the early hours of the morning, waking the patient from sleep. The attacks also occur by day, and may be provoked by the

24. Lewis, Thomas: Heart **15**:305, 1931.

25. Brunton: Lancet **2**:97, 1867; Clin. Soc. Rep. **3**:191, 1870.

26. Hunter: Brit. M. J. **2**:1128, 1909.

27. Powell, in Allbutt, C. A., and Rolleston, H. D.: A System of Medicine, New York, The Macmillan Company, 1910, vol. 6, p. 183 (case 5).

28. Mackenzie, James: Heart **2**:265, 1910-1911.

ingestion of food or by the idea of food. The patients, in relating the symptoms of the attack, frequently speak of strong throbbing of the wall of the chest, rapid and violent beating of the heart and the neck, a feeling of tenseness and a throb invading the head. General pallor is not a feature of the attack; on the contrary, the face may flush, sometimes to a deep suffusion; if pallor comes eventually, it is inconspicuous and confined to the face. A distinctive feature is a change of heart rate and of blood pressure, both lifting to high points. Rapid breathing in the attack is the rule, and is probably a special feature; sweating is the rule, but is not distinctive. It is well known that in many instances of repeated anginal seizure, amyl nitrite fails to bring relief; in the type of case described, the remedy is efficacious, rarely failing to stop the pain. In this connection, it is of particular interest to note that the first case described to illustrate the beneficial action of amyl nitrite, the famous case recorded by Brunton, was of this type. I believe the syndrome to be the usual anginal syndrome at rest in cases of free aortic regurgitation.

Numerous observations on the blood pressure, immediately before and during the attacks, show that the disturbance of the vascular system in the attacks is not provoked by pain, since pain definitely succeeds the rise in blood pressure. The more plausible, though converse, idea that in these cases pain is due simply to the imposing of strain of a given quantity, either distending an irritable aorta or overburdening the working ventricle, likewise fails, for on more than one occasion, after ascertaining the height to which the blood pressure and pulse rate had risen in attacks of pain, equal disturbances of blood pressure and pulse rate have been observed to occur spontaneously, or have been induced by giving exercise, without any trace of pain developing. In other instances, an attack of pain associated with high pressure has subsided, while the blood pressure has remained without appreciable change (or while it has even lifted a little) and the pulse rate has remained as high as before. Putting the matter more generally, it may be stated as a result of direct and careful observation that while attacks are accompanied by a rise of blood pressure and pulse rate, the periods of pain do not correspond with sufficient accuracy to the periods over which pressure and pulse rate equal or surpass what might be regarded as critical levels. Nevertheless, although pain does not provoke the rise of pressure and the high pressure cannot be regarded as the sole determinant of pain, the two phenomena may not be divorced. However, it appears almost necessary to assume that the strict relation between pain and the energy expended is broken by some factor of interference, and the factor in mind is an inconstant state of the coronary vessels. If one accepts the theory of muscular ischemia, then different states of coronary

tone adequately explain discrepancies. If these vessels are involved in the storm and acquire increased tone, then, even if they are not actually constricted, they may fail to carry the extra stream of blood that the heart, beating under an unusual burden, requires of them. If pressure and pulse are raised in some other manner, as by exercise, the increased burden may fall on a heart the coronary flow of which is freer, and thus pain may fail to occur. There is no warrant for regarding the coronary circulation as a purely passive system, the flow through which is governed merely by systemic pressure.[29]

Action of Amyl Nitrite.—In considering why amyl nitrite relieves the pain in anginal attacks, its influence on the blood pressure forms a first enquiry. The effect of the drug on the blood pressure during the anginal seizure itself is alone completely relevant and completely safe to use. Few such observations are available from past records. Brunton,[25] Hunter,[26] Mackenzie [28] and Aubertin and Gambillard [30] all recorded considerable falls in isolated cases, but these few records by no means fully represent the facts. Among a large series of observations for the investigation of this point, I have certainly seen instances in which the blood pressure has fallen considerably after the inhalation, but almost as frequently, if the blood pressure is taken as soon as the patient has lost his pain, the blood pressure is found to present no material change, and in a few instances has actually been found a little raised. Brunton was led to try amyl nitrite for anginal pain because he knew that bleeding relieved this pain, and he believed this relief to be due to a diminished arterial tension. Amyl nitrite, already known to cause vasodilatation, seemed to him the drug that should act similarly; it was natural that, having found it to give relief, he should refer the effects to a fall of blood pressure. Although his conclusion has retained almost universal assent, it has never received sufficient support from immediately relative observations, and facts conflicting with it have now been brought forward. While it is acknowledged that amyl nitrite may lower vascular tension in the attack—and it is granted that in such instances the fall of pressure is possibly adequate to relieve pain—the point to be impressed is that sometimes the fall is inadequate, and that then another explanation must be sought.

29. In an important paper, Feil and Siegel (Am. J. M. Sc. **175**:255, 1928) suggested that transient vascular changes occur in the heart muscle in attacks of angina pectoris, on the basis of observed changes in the form of the electrocardiogram during the attacks. Dr. Feil has recently shown me curves demonstrating these changes in electrocardiograms taken in a case of aortic disease during angina of the type here considered.

30. Aubertin, C., and Gambillard, M.: Bull. et mém. Soc. méd. d. hôp. de Paris **48**:136, 1924.

It seems to have been suggested first by the French clinician Huchard, though on what evidence is unclear, that amyl nitrite may bring relief by opening up the coronary vessels. It has since been shown by experiments on animals that nitrites dilate these vessels.

If one accepts the idea that attacks of anginal pain are brought about by relative ischemia of the muscle, the relief of pain by nitrites may clearly come through a decrease of expenditure of energy, an increase of blood supply or by the joint effects of these two. On the basis of the theory of muscular ischemia, there is no difficulty in understanding why amyl nitrite fails to give relief in the pain of coronary occlusion. Similarly, in angina pectoris there is no difficulty in explaining why amyl nitrite may at times relieve pain without lowering the blood pressure, or why at other times the blood pressure may fall without relief until later. It is also easy to understand why, after the administration of amyl nitrite, the pressure may rise to a high point without causing pain, since the effects of increased coronary flow on the muscle may long outlast the action of the drug. Lastly, it enables one to explain on similar lines why attacks that would otherwise last ten minutes or more are brought to an abrupt and complete end by the inhalation, the direct effects of which are notoriously quite transient.

In this article I have not reexamined the theory of muscular ischemia as a cause of anginal pain exhaustively, but have dealt with those aspects of it that have been given prominence by recent work. This work has brought much support to an old theory, and by more sharply defining it has rendered it more tangible and more capable of proof or disproof.

AURICULAR FIBRILLATION

AMBULATORY TREATMENT WITH QUINIDINE

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In a review of a considerable part of the literature on the use of quinidine sulphate in the treatment for auricular fibrillation, in every instance to my knowledge the patients were hospitalized for treatment. No mention is made of any patient or group of patients who were treated by the ambulatory method. This report deals with twenty-four patients with auricular fibrillation who were treated with quinidine sulphate in the outpatient department of the University of Minnesota.

Since 1918, when Frey [1] introduced quinidine as a therapeutic measure in the treatment for heart disease, much has been written about its efficacy, its toxic effects and the complications resulting from its use. Almost every report in the literature relates instances of the following complications: distressing palpitation, acute heart failure, diarrhea, respiratory or cerebral disturbances and emboli.

There is reason to believe that quinidine has not been given the place it deserves in the treatment for heart disease. Many of the complications that are attributed to quinidine are perhaps due to causes inherent in the patient's condition. The drug is usually blamed immediately when an embolus occurs during treatment with quinidine, especially if the embolus appears after the heart beats become regular, or if the patient dies suddenly during the course of treatment. Death may be due to the drug in some instances, but it is well known that not infrequently accidents occur in fibrillating hearts during treatment with digitalis or without treatment.

Viko, Marvin and White [2] compiled from the literature a group of 484 cases of auricular fibrillation in which treatment with quinidine was given and compared them with 200 cases of auricular fibrillation at the Massachusetts' General Hospital in which this treatment was not given.

Submitted for publication, Sept. 14, 1931.

From the Department of Cardiology, University of Minnesota Medical School.

1. Frey, W.: Ueber Vorhofflemmein beim Menschen und seine Beseitigung durch Chinidin, Berl. klin. Wchnschr. **55**:450, 1918.

2. Viko, L. E.; Marvin, H. M., and White, P. D.: A Clinical Report on the Use of Quinidine Sulphate, Arch. Int. Med. **31**:345 (March) 1923.

They concluded that the danger of embolism under quinidine therapy is not much greater than with any other form of treatment.

Carr [3] reported seventy-seven cases of auricular fibrillation in which treatment with quinidine was given, in twenty-three of which a return to regular rhythm occurred. He stated that in not one of the twenty-three cases did an embolus develop. In reviewing one hundred hospital records of cases of auricular fibrillation, Carr stated that "in sixteen instances there was a history of embolism at some time in the course of the disease."

Levy [4] observed fifty patients with auricular fibrillation whom he divided in two equal groups. He gave the usual treatment for heart failure to group 1 and the usual treatment plus quinidine to group 2. The results were as follows: Among the twenty-five patients treated without quinidine embolus occurred in five; among the twenty-five treated with quinidine embolus probably occurred in one. He stated, too, that the danger of emboli accidents after quinidine has been given undue emphasis."

Korns,[5] in a clinical study of quinidine sulphate in auricular fibrillation, inquired whether:

> . . . the cases of embolism from this cause are more frequent than those which commonly occur in fibrillators who are being treated intensively with digitalis, or is it that we hear more of them by reason of the prominence of quinidin in the medical literature? It is my belief that the actual percentage of such cases will be found almost equally divided between the two causes. We feel it our duty to extend the benefit of digitalis to our patients, although we realize fully the likelihood of subsequent embolism. Similarly, I believe we shall find it our duty to extend the benefit of a normal cardiac mechanism without probability of greater hazard.

Bramwell and Ellis [6] cited the following case: A man, aged 25, with rheumatic carditis of long standing, who was being treated by rest in bed and small doses of digitalis, and who apparently was making good progress, died suddenly. Autopsy did not reveal the cause of death. Bramwell stated that if this patient had been given quinidine, in all probability death would have been attributed to its toxic effects.

Morawitz and Hochrein [7] use quinidine as a prophylactic measure to prevent acute heart failure in decompensated hearts with auricular

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3. Carr, J. G.: The Untoward Effects of Quinidine, Illinois M. J. **46**:445 (Dec.) 1924.

4. Levy, R. L.: Clinical Studies of Quinidin: IV. The Clinical Toxicology of Quinidin, J. A. M. A. **79**:1108 (Sept. 30) 1922.

5. Korns, H. M.: An Experimental and Clinical Study of Quinidin Sulphate: Clinical, Arch. Int. Med. **31**:36 (Jan.) 1923.

6. Bramwell, J. C., and Ellis, R.: The Ultimate Results of Quinidine Therapy in Auricular Fibrillation, Lancet **2**:960 (Nov. 10) 1928.

7. Morawitz, P., and Hochrein, M.: Zur Verhütung des akuten Herztodes, München. med. Wchnschr. **76**:1075 (June) 1929.

fibrillation. They state that since they have adopted this treatment their results have been better.

After considering the aforementioned investigations on the action and results of quinidine therapy, it occurred to me that the unpleasant effects that have been so often mentioned in the literature had been overemphasized, and that a trial of quinidine in ambulatory patients with auricular fibrillation was justified.

Twenty-eight patients were treated by the ambulatory method. In 17 of these 28 cases, or 60.7 per cent, a normal rhythm was restored. If 4 patients who failed to continue treatment are eliminated, sinus rhythm was successfully restored in 70.8 per cent. These results compare favorably with those obtained in many of the hospitalized patients whose cases are reported in the literature. Eismayer,[8] reporting 1,058 cases from the literature up to 1927, found that in 618, or 58.5 per cent, favorable results were obtained. Burwell and Dieuaide [9] reported that in 339, or 55.9 per cent, of 606 cases of auricular fibrillation up to 1923, a return to regular rhythm occurred. More recently Carr,[3] reporting 77 cases of auricular fibrillation, found that in 23 normal rhythm was restored. In 1929, Wolf and White,[10] in reporting 62 cases of auricular fibrillation, found that in 42, or 67.7 per cent, normal rhythm was restored.

DURATION OF RESTORED REGULAR RHYTHM

In our series of seventeen cases the period of time during which the hearts remained regular after they were restored to normal rhythm compares very favorably with the cases reported by Maynard,[11] Harris,[12] Riecker,[13] Viko, Marvin and White,[2] and Wolf and White.[10] Table 1 gives the age of the patients, the total amount of quinidine given, the period of treatment and the duration of the restored normal rhythm. Three of the patients in the series have died since the table was compiled. In one the heart was regular for twelve months; in one, for nine months, and in one, for three months.

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8. Eismayer, G.: Die Behandlung Unregelmässiger Herztätigkeit mit Chinidin, Deutsches Arch. f. klin. Med. **156**:182, 1927.

9. Burwell, C. S., and Dieuaide, F. R.: Clinical Experience with Quinidin, Arch. Int. Med. **31**:518 (April) 1923.

10. Wolf, F. L., and White, P. D.: Auricular Fibrillation, Arch. Int. Med. **43**:653 (May) 1929.

11. Maynard, E. P., Jr.: Five Years' Experience in the Treatment of Chronic Auricular Fibrillation with Quinidine Sulphate, Am. J. M. Sc. **175**:55 (Jan.) 1928.

12. Harris, K. E.: A Series of Cases of Auricular Fibrillation Treated with Quinidine Sulphate, Heart **14**:283 (March) 1929.

13. Riecker: A Clinical Study of Quinidine Therapy, Am. J. M. Sc. **170**:205, 1925.

ETIOLOGY

In relation to etiology, this series of cases was divided into six groups. Table 2 gives the number of cases restored to regular rhythm and the average ages.

TABLE 1.—*Duration of Restored Normal Rhythm*

Name	Age	Total Amount of Quinidine Given, Gm.	Duration of Treatment, Days	Duration of Restored Normal Rhythm, Months
Mr. Os	66	6.6	7	12
Mr. Ov	53	9.4	11	2
Mr. McK	62	12.5	28	7
Mrs. B	31	45.6	35	1
Mrs. Ha	70	24.2	49	10
Mrs. M	62	4.2	14	16
Mr. O'B	69	34.7	54	2
Mr. A	73	19.2	26	3
Mr. W	66	3.8	5	3
Mr. S	61	45.6	61	6
Mr. F	74	18.9	27	2
Mr. B	73	0.6	1	3
Mrs. H	37	76.4	107	7 days
Mrs. M	46	3.0	7	2
Mrs. G	62	2.4	3	Cannot be traced
Mr. L	59	4.8	10	9
Mr. P	67	1.8	7	2

TABLE 2.—*Etiology of Cases of Auricular Fibrillation*

Etiology	Number	Normal Rhythm Restored	Average Age of Those Restored to Normal Rhythm	Average Age of Failures
Rheumatic valvular heart disease	5	4	45.7	26
Hypertension and coronary sclerosis	14	10	63.5	58.5
Hyperthyroidism	1	0	....	..
Hypertension and diabetes	2	1	70	55
Aortitis	1	1	62	..
No apparent heart disease	1	1	73	..

TABLE 3.—*Ages in Decades*

Age	Those Successfully Treated		Those Unsuccessfully Treated	
	Number	Percentage	Number	Percentage
20 to 29	0	....	1	14.3
30 to 39	2	11.8		
40 to 49	1	5.9	2	28.6
50 to 59	2	11.8	2	28.6
60 to 69	8	47.0	1	14.3
70 to 79	4	23.5		
80 to 89	0	....	1	14.3

The youngest patient, Mrs. F., aged 26, who had a rheumatic valvular heart disease, failed to respond to quinidine.

Over 80 per cent of the patients successfully treated were 50 years of age and over. In the unsuccessful group three were below the age of 50 and four were over the age of 50. It has been shown by several investigators that age is not an important factor for the successful action of quinidine.

SEX

In this series fifteen were males and nine, females; of the seventeen in whom the heart became regular, twelve were males and five, females. Of the patients in whom failure occurred, four were females and three, males. The heart beat became regular in twelve of the fifteen males, or 80 per cent, and in five of the nine females, or 55.5 per cent.

DURATION OF FIBRILLATION

In all but one of the cases studied chronic fibrillation occurred. In Mr. B., aged 73, an auricular fibrillation developed after the injection of a foreign protein for arthritis. Fibrillation had been present for two days, and 6 grains (0.4 Gm.) of quinidine sulphate (2 grains [0.13 Gm.] every two hours for three doses) restored the heart to regular rhythm. In more than 60 per cent of the cases, fibrillation had been present for more than one year. In two cases fibrillation had been present from ten to twelve years. The rhythm of the heart beat in one of the latter became regular.

METHOD OF TREATMENT

All patients are first given digitalis, preferably for a few days. If the heart is decompensated the patient is well digitalized until there is evidence that the heart is fully compensated, or nearly so. Quinidine is then given in small doses. The first day 0.1 Gm. is given; the following day 0.2 Gm., and the third day 0.4 Gm. The patient is given 0.4 Gm. per day for five days. Every two hours 0.1 Gm. is taken. At this point the dose of digitalis may be reduced or discontinued, depending on the degree of cardiac compensation and the heart rate. On the seventh day the dose of quinidine is increased to 1 Gm. per day, 5 grains (0.324 Gm.) every two hours until three doses have been given. After a few days, if necessary, the dose is increased to 20 grains (1.3 Gm.), 5 grains being given every two hours until four doses have been administered; then the dosage is increased to 30 grains (1.95 Gm.), 10 grains (0.65 Gm.) every two hours. As much as 40 grains (2.6 Gm.) per day was given in 10 grain (0.65 Gm.) doses at two hour intervals. As soon as the rhythm of the heart becomes regular the dose of quinidine is reduced, and a maintenance dose is established. This amount may be about 10 grains per day for several days, then 5 grains in single doses. Most of the patients are given 5 grains daily, and many are given 5 grains every other day.

In the series treated quinidine was seldom discontinued when the patient complained of the following symptoms: slight headache, diarrhea, nausea or vertigo. With the possible exception of one case, in no

instance were the toxic symptoms of quinidine so pronounced that the drug had to be discontinued.

FAILURES

In seven cases, including two in which accidents occurred, the heart failed to be restored to normal rhythm.

CASE 1.—*History.*—Mr. R., aged 49, reported to the dispensary on Oct. 27, 1930, complaining of shortness of breath, swelling ankles, vertigo, cough, hemoptysis and pain in the neck and in the upper right quadrant of the abdomen. He had been in the University Hospital and the Minneapolis General Hospital three times since 1928, each time from one to three months. The previous history was irrelevant except that he had had "rheumatic fever" at the age of 26.

Examination.—Physical examination showed slight pitting of the skin of the legs. The liver was enlarged about 4 cm. below the costal margin; it was soft and slightly tender. The heart was enlarged; an orthodiagram showed: transverse diameter of the heart, 19.1 cm.; of the chest, 28.5 cm. The heart rate was irregular; the apical rate, 100; the radial rate, 84. The vital capacity was 2,300 cc., 68 per cent of the calculated norm. The blood pressure was 180 systolic and 100 diastolic. An electrocardiogram showed arborization block and auricular fibrillation. The patient was given 1 cc. of tincture of digitalis three times daily, and he reported back on October 29 improved.

Course.—The patient was told to continue to take digitalis and to take 0.1 Gm. of quinidine that afternoon (October 29), 0.2 Gm. the next day and 0.3 Gm. the third day. That night, a severe attack of pain over the precordium and marked dyspnea developed. The patient was sent to the Minneapolis General Hospital, where he died the next day. The diagnosis at autopsy was hypertrophy and dilatation of the heart, coronary thrombosis and myocardial infarction. Microscopically, the material in the coronary vessels was thrombosed; no mural thrombi were found. This death can hardly be attributed to quinidine. It was the opinion of the pathologist that it was only a coincidence that death occurred after the administration of the drug.

CASE 2.—*History.*—Mrs. H., aged 37, had not been well since October, 1929, when she had had pneumonia. She had been in the University Hospital for five weeks in 1929 and again in 1930 for heart disease. On Aug. 9, 1930, she came to the dispensary complaining of dyspnea on exertion. The patient gave a history of rheumatism at the age of 16.

Examination.—Examination of the heart showed it to be of a double mitral type, and was enlarged. An orthodiagram showed: transverse diameter of the heart, 14.2 cm.; of the chest, 25.2 cm. An esophagram showed a definite displacement of the esophagus by the left auricle. The apical pulse rate was 116; the radial pulse rate, 104. The blood pressure was 150 systolic and 106 diastolic. The patient was given digitalis until Sept. 22, 1930, when quinidine sulphate was added.

Course.—Small and increasing doses of quinidine were given until Jan. 7, 1931, when the pulse was regular for the first time. An electrocardiographic report showed 66 beats per minute, regular, and delayed auriculoventricular conduction.

The heart rhythm was regular until Jan. 14, 1931, when I was called to the patient's house. There was complete paralysis of the right side of the face, arm and leg. The pulse was totally irregular. I do not know for how long it had been irregular or whether the hemiplegia occurred before the heart rhythm became irregular or afterward.

SUMMARY

1. Twenty-eight cases of auricular fibrillation in which the patients were treated with quinidine are reported; four did not continue treatment, so that only twenty-four were treated satisfactorily by the ambulatory method.

2. In seventeen cases, 70.8 per cent of the twenty-four in which treatment was satisfactory, normal rhythm was restored.

3. Of the successfully treated patients, four had rheumatic valvular heart disease; ten had hypertension and coronary sclerosis; one had diabetes and hypertension; one had syphilitic aortitis, and one had an apparently normal heart.

4. Eighteen of the twenty-four patients were 50 years of age or over; in fourteen of this group, or 77 per cent, the heart was restored to regular rhythm. In three of the remaining six patients regular rhythm was restored.

5. The group with hypertension seemed to respond more quickly than the rheumatic group.

6. In all but one there was chronic fibrillation for from two months to ten or twelve years.

7. In the treatment small doses of quinidine were given to start with; then the dose was gradually increased.

8. Tincture of digitalis was always given first or in conjunction with quinidine during treatment.

9. Two accidents occurred during treatment: one death from coronary thrombosis and a case of hemiplegia.

ENCEPHALOPATHY DUE TO CHRONIC PLUMBISM

REPORT OF A CASE

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Since the monumental work of Aub and his co-workers [1] on lead poisoning, all reports of cases seem dwarfed into insignificance. However, the following reasons justify the case herewith published:

1. Good results were obtained from mobilization treatment.

2. "Deleading" resulted in a syndrome similar to tetany on two occasions, in each of which intravenous administration of calcium was immediately and spectacularly effective.

3. The case was not associated with hypertension.

REPORT OF A CASE

History.—L. H., aged 48, a plumber, complained of inability to coordinate, particularly in walking. His wife said that he was mentally confused and irritable. He had worked as a plumber many years and had handled considerable red lead. He had also been in many new houses where painting was being done, and had worked a good deal with boiling lead. Therefore, there was a possibility of poisoning from the gastro-intestinal tract, or by droplets and fumes from the respiratory tract.

Until four years before examination the patient had been very well, but at that time he was seized with severe cramps in the abdomen, vomiting and diarrhea, which was symptomatic of lead colic, though a diagnosis was not made. Presently the pain, vomiting and diarrhea disappeared, but he began to have pain in the muscles, which he thought was due to "rheumatism." The muscular pains had continued. They were worse in the calves of the legs and in the back. The patient also noticed that during the four years previous to examination, he had dropped his tools frequently, and had not been able to use them with his usual skill.

There was a gradually increasing weakness of the legs which became nearly a paralysis in November, 1926 (seven months before examination), and about the same time the patient noticed severe headaches and twitchings of the arms and legs, greatly aggravated by fatigue. There has been marked propulsion and a very decided tendency to fall to the left side. This twitching progressed gradually until he was unable to walk. He was very drowsy and yawned interminably. His wife stated that he was very irritable and disagreeable, and that his memory was extremely poor. He was immensely confused, and at times completely disoriented.

Submitted for publication, July 15, 1931.

This case was presented before the New Haven Medical Association in June, 1928.

1. Aub, J. C., and others: Lead Poisoning, Medicine **4**:1, 1925.

There was some blurring of the vision, which has gradually cleared up until he was able to see well. His appetite had been poor, and he had been constipated.

There had been nycturia once a night and once he passed some blood. He had a great deal of difficulty in starting the urinary stream.

The past history was irrelevant, except for work as a plumber. The patient had been an extremely strong man, able to beat all his companions in rough and tumble fights. His weight had been stationary, and his habits good. He said that he had never had a venereal disease.

He had been to a number of physicians who considered various diagnoses including brain tumor, epidemic encephalitis, syphilis and autointoxication. He was seen at the Yale Mental Hygiene Clinic, and a diagnosis of lead encephalopathy was made by Dr. C. C. Fry. Potassium iodide was given for four days for elimination of lead. Lead was found in the stools and urine.

Physical Examination.—The patient appeared well developed and well nourished. There was no cyanosis or dyspnea. The skin showed a very pasty color. The temperature was 98 F.; pulse rate, 76; blood pressure, 120 systolic and 80 diastolic, and weight, 170 pounds (77.1 Kg.).

The eyes reacted to light promptly. The eyelids showed no ptosis. The eyegrounds were normal.

The teeth showed some caries and several were loose. There was a "lead line" on the gums. Roentgenograms of the teeth were negative. The tonsils were small with no pathologic process. The neck was normal.

The heart was slightly enlarged to the left, and the second aortic sound was rather loud.

The lungs showed numerous small râles rather evenly distributed. No other signs were present.

The abdomen was normal, and there was no tenderness or masses.

The extremities were normal.

The reflexes were all exaggerated, but all were present and about equal.

The blood vessels were not palpable.

The glandular system was normal.

Marked weakness was noted in all muscles, particularly in the calves and thighs. The patient could not walk, and if left alone fell to the left or forward. He could not even sit up by himself.

He was mentally confused, and did not remember the day or the month. He remembered very little of what happened the day before, was irritable and fell asleep while I was taking the history.

Laboratory Examination.—Lead was found in the urine and feces, at the New Haven Hospital. Hemoglobin was 85 per cent; red blood cells numbered 4,500,000, white cells 6,000 and polymorphonuclears 65 per cent. The smear showed no stippling or secondary anemia. The Wassermann reaction of the blood was negative. Routine examination of the urine gave negative results except for a slight trace of albumin which later cleared up. The spinal fluid showed: cells, 25; colloidal gold curve, 00004555430; phthalein, 75 per cent; nonprotein nitrogen, 29 mg.; Wassermann reaction, negative, and globulin, negative. Lead was not present.

Treatment.—Syrup of hydriodic acid, 4 cc. (1 fluid drachm), was given every three hours for four days. Lead was now found in the feces and urine. After four days this treatment of elimination was discontinued. Mobilization was attempted by the use of calcium chloride and milk (calcium lactate, 1 Gm. twice a day and 2 quarts of milk a day).

For the first three weeks improvement was slow but steady. The mental symptoms began to clear, and the patient's memory returned slowly. There were

five days of regression and then, suddenly, in the fifth week of the treatment he got up out of bed one morning, shaved himself and began to walk. After that he continued to walk by himself, and his memory was almost normal. I considered that I had mobilized the lead in the bones, at this point, as trilead phosphate.

In May, 1927, the "lead line" was absent. There were no stippled cells. The hemoglobin was 80 per cent, and the red blood cells 5,000,000. The urine contained no lead.

When the patient began to be fatigued, twitching of the muscles occurred, but at all other times he had no more subjective symptoms.

From May, 1927, until January, 1928, the patient continued doing well without episodes of any kind. The urine and feces were clear of lead on several occasions. The patient continued to gain strength and coordination, so that by fall he was able to do all types of work. Owing to the fact that the change in the hydrogen ion concentration might be induced by some casual illness as acute tonsillitis, grip, pneumonia or the like, it was felt that the man should be "deleaded." It seemed that "deleading" by the use of diet and drugs would be more simple to control than to attempt to cope with a sudden "deleading." A diet very low in calcium, which omitted milk, eggs, green vegetables and fruit and consisted of meat, liver, potato, rice, tomato, canned corn, bananas, apples, tea, coffee and all foods prepared without milk, such as salt-free nephritic bread, sodium bicarbonate bread or crackers, was prescribed. Sugar, salt and pepper were allowed. With this diet ammonium chloride, 1 Gm., ten to twelve times a day in a glassful of water was also given. The diet proved very efficacious in "deleading," for after three days of the diet and medication lead was found in the urine and feces. The patient was discharged from the hospital after about two weeks of the treatment and went home, continuing the diet with ammonium chloride until February 25, when the ammonium chloride was discontinued. During that period, he had no toxic symptoms, and lost that "aching feeling" in his muscles and joints.

Course.—On March 10, 1928, the patient was feeling better than he had felt any time in the past six months. Four examinations of the urine between January, 1928, and March, 1928, showed no lead.

On March 10, the patient went to a club meeting and ate pickles, cheese, fresh peaches, rye bread and drank beer and wine. He drove his car to and from the meeting; a friend who went with him thought he acted a bit peculiarly. He went to bed and in the morning about 6 o'clock, his wife noticed that he was breathing heavily. Thinking that it was merely a sound sleep, she did not wish to disturb him, but on entering the room at 9 o'clock, she found him in the midst of a convulsion and immediately called me. It was about an hour and a half before I could reach the patient's home. When I arrived, he had already had eight convulsions and some probably previous to the time that his wife entered his room. A physician who had been called in the interim was giving him a hyperdermic of morphine. The convulsions were severe, tonic and clonic convulsions, and were followed by a long period of apnea during which time he became unbelievably blue, although the pulse stayed fairly strong except during one convulsion when it became weak and thready.

Each convulsion began with twitching of the muscles of the eye on the right side resulting in an upturning of the lids and proceeded to involve the entire body. The pupils were widely dilated in spite of the use of morphine and did not react to light. The eyegrounds showed constriction of the retinal arteries, but otherwise were normal. The eyes were turned upward and inward. These convulsions were followed by periods of apnea, one of which measured nearly three minutes by the watch. During these periods, artificial respiration was given. During one of the

early convulsions, he had bitten his tongue badly and the bed was covered with blood. There was evidently also incontinence of both feces and urine during one of the convulsions. Between convulsions, Chvostek's and Trousseau's signs could be elicited easily. The eyegrounds were normal. Physical examination revealed no lesion in the lungs or heart, nor, so far as we could perceive, in the abdomen. The blood pressure was 110 systolic and 70 diastolic. Serum calcium was 7.5 mg. and nonprotein nitrogen 32.

I went immediately to Grace Hospital and obtained 60 Gm. of calcium chloride ready for intravenous administration. This was given to the patient intravenously. Following this the stomach was washed out, and in the contents we recovered the remains of pickles which he had eaten the night before. Following this procedure, a pint of milk was left in the stomach by gavage. A spinal puncture was done, and although the fluid was under pressure it was negative for globulin and for cells, and the Wassermann reaction was negative. Colloidal gold also proved normal. Fifteen cubic centimeters of fluid was removed. Sixty units of parathyroid hormone was also given intravenously with the idea that possibly the calcium might be more quickly utilized by the use of the hormone. He also had two doses of $\frac{1}{150}$ grain (0.4 Gm.) of hyoscine hydrobromide.

Following the intravenous administration of calcium and the parathyroid hormone the patient had three convulsions, the first of which was as severe as those he had preceding this medication; the last two were noticeably less severe; he also had three very mild convulsions. From 1 o'clock on he had no more convulsions.

He was moved to Grace Hospital by ambulance, at 4 p. m. He started to move his arms purposefully about 6 in the evening, and at 9 he was able to make it evident by signs that his bladder was distended. He was catheterized and 1,500 cc. of urine obtained. This specimen did not contain lead and was normal in all respects.

The following morning he was able to speak, but thickly, because of the lacerated and swollen tongue, but it was evident that he understood perfectly everything that was said and could have spoken clearly except for the injury to his tongue. He remembered what had happened two nights previously, but nothing of what happened the previous day. He had a temperature for a few days, which was assumed to be due to the infection in his tongue, because as soon as the infection cleared up the temperature disappeared. Physical examination at that time gave negative results, except for ptosis of the left eyelid and the bitten tongue. The blood pressure was 115 systolic and 70 diastolic. The patient was put on calcium lactate and made an uneventful recovery.

In September, 1928, he was feeling better than at any time in the past five or six years. The pains were completely gone from his muscles and bones. He had no incoordination or confusion, and his personality was what his friends claim it used to be.

From the attack of convulsions in March, 1928, until September, 1928, the patient's condition was good. He was able to do ordinary labor such as chopping wood, to complete his inventory, to do accounting in his business and to drive his automobile.

From September, 1928, until December, 1928, the patient's condition became gradually less good. He was seen at various intervals with steadily increasing neurologic signs. Headaches became a permanent feature. They were severe and explosive, beginning with dulness and reaching a crescendo. At these times he was confused, and memory was difficult. The reliefs from the headaches were sudden, and they were usually improved by rubbing the back of the neck. At vari-

ous times following these headaches, he showed right-sided hemianopia. The blood pressure had been low from September to December, 1928, being around 110 systolic and 70 diastolic to 125 systolic and 75 diastolic. The nonprotein nitrogen had been 30; the plasma chloride, 550.

About December 14, the patient had an acute diarrheal disease for which he did not call me. Four days later, December 18, I was called. For the past four hours the patient had had convulsions similar to those that occurred in March, 1928. They began with twitching of the muscles of the right eye, resulting in the upturning of the lids and deviation of the eyes and head to the right. The pupils were widely dilated during the periods of convulsion. The periods of apnea were of about the same length as those in March, and between the convulsions artificial respiration was given. Between the seizures, speech was difficult, sometimes amounting to complete aphasia. The eyegrounds showed constriction of the retinal vessels. No evidences of meningeal irritation were noticed. Cyanosis was as deep as in March. The blood pressure was 120 systolic and 70 diastolic. The heart and lungs were normal. Nonprotein nitrogen was 32; plasma chlorides, 560, and serum calcium, 6.5 mg. The urine was normal. These attacks endured for twenty-four hours in spite of treatment.

The diagnosis at this time was a degenerative process in the brain; directly or indirectly, the convulsive seizures seemed related to the calcium deficiency in the blood. Consultation with Dr. James Fox corroborated these findings, and he gave his impression:

"The condition is a degenerative process in the brain. The nature of vascular occlusion is directly associated with the chronic plumbism which is thought to be responsible for the clinical picture." The chief irritative lesion was assumed to be in the left hemisphere. In view of both the irritative and paralytic phenomena to the right side, large sedative and antispasmodic medication was advised, including inhalants (ether and chloroform). This treatment was carried out without beneficial effect.

The following day, as treatments had been ineffective for forty-eight hours, and since the serum calcium was so low, intravenous injection of calcium, 60 grains (3.9 Gm.), was given, purely empirically, because it had accomplished such spectacular results in March, although the patient was at this time on a high calcium diet. The result was as spectacular this time as it had been in March, 1928.

The patient made a complete recovery from the convulsions shortly after the calcium was given.

In looking backward on the case, it seems that the cause of these convulsions was tetany just as in March, 1928, and at this time it was a case of tetany due to inability of an inflamed intestinal tract to absorb calcium even with the patient on a high calcium diet. Examination of the spinal fluid obtained at this time was negative in all respects. Urine obtained at this time showed no lead.

Following the convulsion in December, the patient finally recovered partially, but never was able to do more than walk about the house, attend to his own habitual needs and occasionally go for a ride in the automobile or to shave. The improvement was slow, but apparently there was an upward trend to it. Roentgenograms of the teeth were made and found to be negative. The eyegrounds showed paralysis of the muscles and slight pallor of the optic disks. The patient was seen by Dr. Arthur Alling, who confirmed these findings. In June, 1929, he was at the point where he could be roused with difficulty and only for a short time. He was then seen by Dr. George Blumer, who corroborated the diagnosis of multiple central nervous system lesions due to lead.

From June to September, the patient made a slow but steady improvement. In September, 1929, he developed what was apparently severe bronchitis and general infection of the upper respiratory tract. I was away at this time, and Dr. Israel Blodinger cared for him during my absence.

During this attack, the patient lost a tremendous amount of ground. He lost a great deal of weight, and when next seen in November, 1929, found it impossible to walk, because of incoordination and weakness of the muscles. It was difficult for him to swallow food, and the act was very slow. This was probably due to muscular weakness and incoordination. He was very stuporous and could hardly be aroused. He showed all of the neurologic manifestations which were noted before, and at this time none were transitory. He was seen in November by Dr. Joseph Aub, of Boston. Dr. Aub's diagnosis was also chronic lead poisoning with multiple lesions of the central nervous system.

On the basis of the past history and findings, Dr. Aub recommended that intravenous calcium therapy be tried again, considering that perhaps the man was unable to absorb calcium even though he was getting fair doses of it into the stomach. This suggestion was carried out but he did not improve. Bronchopneumonia developed and he died with high temperature, increased pulse rate and respiratory rate, after a few days of treatment.

Autopsy was refused in spite of every effort.

COMMENT

The diagnosis in the case seemed very clearly to be lead poisoning. Lead was found in both feces and urine. The encephalopathy was apparent in the history and physical examination. In the light of subsequent events, it was considered possible that there may have been multiple sclerosis all the time. But this does not seem tenable, particularly as rational treatment for lead poisoning removed all symptoms for a time. That multiple lesions of the spinal cord and brain later developed is perfectly apparent, but I believe they were caused by lead poisoning, itself.

The reason for these attacks of convulsive and explosive nature is not clear. Certainly there is no evidence of arteriosclerosis in the general physical examination, nor in the examination of the eyes, and there was no hypertension. Examination of the urine showed normal concentration and the blood chemistry was normal throughout the course, the nonprotein nitrogen and plasma chloride figures were normal. Therefore, uremia cannot be said to explain the picture.

During the discussion before the New Haven Medical Association it was suggested that acidosis might account for the convulsive seizures, but there was no ketonuria or other findings at these times suggestive of acidosis. In addition to these negative findings, the following signs of tetany could not be explained by a simple acidosis. Apparently the calcium depletion seemed to have some direct or indirect relationship to the state.

1. The patient was on a low calcium diet at the time of the first seizure.

2. At the time of each seizure, the serum calcium was low.

3. On both occasions, Chvostek's and Trousseau's signs were present.

4. Spectacular and immediate results were obtained from the use of intravenous calcium.

That there was a temporary increase in intracranial pressure would seem evident from the fact that the spinal fluid was under marked pressure at both times of seizure. Probably there was a marked cerebral edema similar to that described by Blackfan,[2] Rees,[3] Volhard[4] and others.

Vasoconstriction with temporary cerebral anemia was probably the direct cause of the convulsive seizure and resulted from cerebral edema. This symptom complex has been noted by various observers—Elschnig,[5] Wagener,[6] and Smith[7]—who in studying the eyegrounds during convulsions found constrictions of the retinal vessels similar to that which was found here. Although such authorities as Traube[8] and Vaquez[9] stated that arterial hypertension is always present in lead encephalopathy, it was not found in this case, nor was there present any crescendo increase in blood pressure, such as was described by Oppenheimer[10] and Fishberg, just previous to the convulsive seizures.

It is also noteworthy that the patient did not die in a convulsive state. His course was gradually downward for the period of a year after the last convulsive state, and he died of bronchopneumonia, with evidence of marked degenerative lesions of the brain and spinal cord. The question of the relation of treatment to the convulsive seizures is interesting, but uncertain. It is apparent that lead mobilization was effective for the man made nearly a complete recovery during the treatment. The "deleading" was successful as considerable quantities of lead were poured out in the urine during the process, and then came to a period, during treatment, when no lead appeared in the urine.

2. Blackfan, K. D.: Bull. Johns Hopkins Hosp. **39**:69, 1926.

3. Rees: On the Nature and Treatment of Diseases of the Kidney, London, 1850, p. 67.

4. Volhard: Der arterielle Hochdruck, Verhandl. d. deutsch. Gesellsch. f. inn. Med. **35**:134, 1923.

5. Elschnig: Wien. med. Wchnschr. **48**:1305, 1898.

6. Wagener, reference deleted.

7. Smith: Brit. M. J. **2**:1380, 1909.

8. Traube: Ges. Beitr. z. Path. u. Phys. **2**:551, 1871.

9. Vaquez: La tension artérielle dans le saturnisme aigu et chronique, Semaine méd. **23**:385, 1904.

10. Oppenheimer, B. S., and Fishberg, A. M.: Hypersensitive Encephalopathy, Arch. Int. Med. **41**:264 (Feb.) 1928.

The "deleading" treatment was apparently carried further than was desirable in that it produced a calcium depletion and led directly or indirectly to the convulsive seizures in the first instance. However, the complete recovery of the patient from this state and the absence of all symptoms for a long period after this incident appear to indicate that this was not contributory to his demise.

The second convulsive state could not be attributed to treatment as the patient was at the time on a high calcium diet and therapy, although it is probable that a diarrheal disease made it impossible for him to utilize the calcium ingested. His recovery from the second convulsive state was complete so far as tetany was concerned, and he never showed any further symptoms of this condition.

In the light of the entire findings in the case it would seem that the patient died of a gradually increasing encephalopathy caused by chronic plumbism. The early central nervous lesions of this condition gradually progressed and were the cause of death.

SUMMARY

1925, summer: The patient had an attack of lead colic.

1925-1927: Increasing muscle weakness and pains of myositis were noted.

1926, November: Symptoms which had been present to a slight degree for a long time were aggravated, i. e., loss of appetite, mental confusion, loss of memory, disorientation, irritability, loss of coordination, particularly in walking, drowsiness, vertigo and headache.

1926, December: Bloody urine was passed and from then until April the patient found it difficult to start the urinary stream. Albumin was present throughout this period but then cleared.

1927, April: Physical examination and laboratory findings showed lead poisoning. (November and December notes.)

1927, May: The patient's mental condition cleared up, and most of the symptoms and physical signs of lead poisoning disappeared. All laboratory findings were negative.

May to July: There was disappearance of all symptoms except muscular pains, the patient stated that he "felt lead in his bones."

July to January, 1928: The patient gained in strength so that he was able to do quite heavy work but still had muscular pains.

January: "Deleading" was done at Grace Hospital. A low calcium diet with ammonium chloride in large doses was given; lead was again found in the urine at this time for a period of ten days. No episodes of any kind occurred.

March 10: For the first time the patient felt entirely well. The muscular pains disappeared. Four examinations of urine and feces were negative for lead.

March 11: Convulsions with apnea, coma and tetany occurred. These were promptly relieved by the intravenous administration of calcium and parathyroid hormone. The patient was again sent to Grace Hospital. He was put on a high calcium diet, and given calcium lactate by mouth.

March 18: The patient's mental condition cleared up, and he said that he felt well in every respect. Friends corroborated this statement.

December: The patient had a diarrheal disease for which he did not call me. Five days later, the convulsions, apnea, coma and tetany again developed. All types of sedative, including ether, were used but did not avail. Intravenous calcium again was effective. The patient recovered in part but not entirely. He showed some right-sided muscular weakness for about one week, and then this cleared. He was able to tend to his personal needs.

December to May, 1929: There was a slow but steady improvement. The patient was able to get out in his car.

May to June: There was a period of decline, so that in June the patient could only be roused with difficulty. He was still on calcium therapy.

June to September: There was a slow but steady gain, so that the patient was better than in June but not yet able to do much more than ride in a car.

September: He had an acute disease of the upper respiratory tract. He was under the care of another physician and lost a great deal of ground.

November: He never recovered completely from the infection in September, and late in November he developed bronchopneumonia and died.

SPONTANEOUS NEPHRITIS IN THE RABBIT

CHANGES IN THE URINE, BLOOD METABOLITES, PHTHALEIN EXCRETION AND BLOOD PRESSURE IN A GROUP OF TWENTY ANIMALS FOLLOWED FOR A PERIOD OF THREE YEARS

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During the hundred and twenty years from 1800 to 1920, there was in the United States a gain of twenty-five years in the average expectancy of life. In the two decades from 1900 to 1920, there was a gain of five years. The average expectancy of life at the present time is 55.33 years for white males, and 57.52 years for white females. The chief statistical cause for the increase in life expectancy has been the saving of infant and child lives, but there has been an actual increase in the life expectancy of adults. As a result of the increase in the average span of life, a larger number of persons are entering the later decades when the so-called degenerative diseases take their toll. These diseases are chronic nephritis, degenerative heart conditions and hypertension. These three groups, which are closely related in both an etiologic and a pathologic sense, were responsible for 45.5 per cent of the deaths of persons beyond 45 years of age in the United States during 1920.[1] It is evident that a further prolongation of the span of life must concern itself with a delay in the onset of degenerative diseases and a prolongation of their course in those who have already developed them.

Since the classic descriptions of Bright in 1827, the etiology of chronic renal disease has challenged increasing attention. Of the numerous theories, that which postulates renal damage as resulting from a long-continued, high protein intake has attracted most attention, in part because protein catabolites are excreted almost entirely by the kidney. An extensive literature has accrued both for and against this thesis.

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1. Statistical Bulletin, Metropolitan Life Insurance Company **8**:1 (Nov.) 1927.

Experimental results have been conflicting. Osborne, Mendel and their associates [2] fed diets, of which protein contributed two thirds of the total calories, to rats for as long as four hundred days. Wheat, corn and casein were used. The kidneys of these animals were uniformly hypertrophied and the tubules dilated, but no true nephritis was produced. Jackson and Riggs,[3] in experiments of a similar nature, fed a 76 per cent casein diet to rats for as long as twenty months and reported negative results. The experiments of Addis, MacKay and MacKay,[4] in which diets of 69.5 per cent protein were fed to rats over a period of one third natural life, were in apparent confirmation of these results. Recently, Parsons, Smith, Moise and Mendel,[5] in an investigation primarily of the effect of the protein content of the diet on the reproductive cycle of the rat, found that a high protein content, even after unilateral nephrectomy and the added burden of gestation and lactation, caused only physiologic hypertrophy of the kidneys. Similarly negative results have been reported by Anderson,[6] who fed rabbits a diet of from 55 to 60 per cent beef protein for periods as long as one year. In some of the animals varying amounts of renal tissue were removed. Hypertrophy of the kidneys or kidney remnants resulted, but no evidence of nephritis was discernible.

On the other hand, experiments in which the results were strikingly positive have been reported. Moise and Smith [7] regularly produced renal lesions in rats as soon as ninety days after unilateral nephrectomy when fed a high protein diet. The nephritis was apparently of a diffuse type. Polvogt, McCollum and Simmonds,[8] by diets entirely sufficient for growth and maintenance and deficient only in containing from 31 to

2. Osborne, T. B.; Mendel, L. B.; Park, E. A., and Winternitz, M. C.: Physiological Effects of Diet Unusually Rich in Protein or Inorganic Salts, J. Biol. Chem. **71**:317 (Jan.) 1927.

3. Jackson, H. J., Jr., and Riggs, M. D.: Effect of High Protein Diets on Kidneys of Rats, J. Biol. Chem. **67**:101 (Jan.) 1926.

4. Addis, T.; MacKay, E. M., and MacKay, L. L.: The Effect on the Kidney of the Long Continued Administration of Diets Containing an Excess of Certain Food Elements, J. Biol. Chem. **71**:139 (Dec.) 1926.

5. Parsons, H. T.; Smith, A. H.; Moise, T. S., and Mendel, L. B.: Reproduction and Excess Protein in the Diet, Arch. Path. **10**:1 (July) 1930.

6. Anderson H.: Experimental Renal Insufficiency: Effects of High Protein Diet in the Presence of Low Renal Function on the Kidneys, Aorta, and Liver; Changes in the Blood Pressure and Concentration of Blood Metabolites, Arch. Int. Med. **37**:297 (March) 1926.

7. Moise, T. S., and Smith, A. H.: The Effect of High Protein Diet on the Kidneys, Arch. Path. **4**:530 (Oct.) 1927.

8. Polvogt, L. M.; McCollum, E. V., and Simmonds, N.: The Production of Kidney Lesions in Rats by Diets Defective only in That They Contained Excessive Amounts of Proteins, Bull. Johns Hopkins Hosp. **34**:168 (May) 1923.

41.3 per cent protein, regularly produced casts and intense congestion of the kidneys. They regarded the high protein diet as nephrotoxic.

Newburgh and his co-workers,[9] in carefully conducted experiments over long periods of time, have produced renal lesions of a degenerative character in both albino rats and rabbits by using diets rich in casein, liver,[9a] beef, egg white and soy bean. They concluded that the severity of the lesion depended more on the duration of the experiment and the nature of the protein than on its amount. Urea added to the diet was innocuous, which suggested that the nephropathy was not the result of a nonspecific increase in the nitrogen metabolism. The renal lesion was primarily confined to the tubules. However, in the rat, a 72 per cent beef protein diet caused definite glomerular changes. Similar results in the rabbit have been reported by other observers[10] using a casein diet.

In previous experiments,[11] we have been able to confirm these positive results. On using both animal and vegetable protein (liver diet, 40 per cent protein; oat diet, 16 per cent protein; soy bean diet, 38 per cent protein) in feeding experiments with rabbits extending over a period of two years, we found a high incidence of chronic nephritis and arteriosclerosis. This nephritis was characterized by persistent urinary abnormalities, retention of nitrogen end-products in the blood and elevation of blood pressure.

On examining these data in retrospect, it was thought that the experiments could be criticized on the ground that the pathologic alterations might have arisen spontaneously. A study was therefore undertaken to answer the following questions:

1. Are spontaneous renal lesions of common occurrence in the rabbit?
2. Are they distinguishable pathologically from experimentally produced lesions?
3. Are they of sufficient magnitude to cause disturbances in the body economy of the living animal?
4. Will an experimentally fed high protein diet exaggerate a spontaneous nephritis?

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9. Newburgh, L. H., and Curtis, A. C.: Production of Renal Injury in the White Rat by Protein of the Diet, Arch. Int. Med. **42**:801 (Dec.) 1928. Newburgh, L. H.: The Production of Bright's Disease by Feeding High Protein Diets, ibid. **24**:359 (Oct.) 1919.

9a. Newburgh and Johnson, in a recent paper (J. Clin. Investigation **10**:153, 1931), stated that a 20 per cent sodium nucleate diet is nephrotoxic in less than a year. The sodium nucleate was prepared from liver, but the changes produced were qualitatively different (fibrous tissue proliferation, changes of the arteriolar walls) from those that result from feeding liver. It is concluded that liver contains forms of nonprotein nitrogen other than purines which are nephrotoxic.

10. Maclean, H.; Smith, J. R., and Urquhart, A. L.: Effect of High Protein Diet on Renal Function, Brit. J. Exper. Path. **7**:360 (Dec.) 1926.

11. Nuzum, F. R.: Changes in the Kidney in Animals with Increased Blood Pressure While on High Protein Diets, Arch. Int. Med. **40**:364 (Sept.) 1927.

We have previously described two distinct pathologic types of spontaneous renal lesions encountered in the routine histologic examination of the kidneys of a hundred and ninety supposedly normal animals.[12] The first type was a focal scarring which gave no evidence of its presence during life and which occurred only twice (an incidence of 1 per cent) in this group of animals. It may be disregarded. The second type was very different. It was a diffuse lesion, involving to some extent all of the renal structure. It also occurred only twice in this group of one hundred and ninety normal animals. However, in a group of twenty animals comprising the present study, which were selected during life because of persistent urinary abnormalities, this diffuse nephritis was the pathologic change. It was morphologically indistinguishable from the nephritis that we had previously produced by feeding high protein diets. In the present study we wish to trace the development of this spontaneous renal lesion in these twenty animals. It will be shown (1) that the clinical and laboratory features are similar to those characterizing the nephritis produced by high protein diets, and (2) that the feeding of a high protein diet to animals already having this lesion will increase the renal damage.

From a total of two hundred and fifty rabbits, twenty full grown animals were selected because of the repeated presence in their urine of albumin, casts and cylindroids. They varied from 6 months to 1 year in age, and were kept in outdoor hutches the year around. They were observed for a period of thirty-six months.

During the first year the animals were fed a diet of barley, 85 Gm., and alfalfa, 50 Gm., daily, with the addition of other greens twice each week. This diet was so balanced that the reaction of the urine of these animals was neutral or slightly alkaline, the p_H averaging 7. During the second year of the experiment the p_H averaged 8.5, owing to the fact that it was necessary to feed alfalfa ad libitum to the animals to keep them in good condition. During the third year, ten of the animals were placed on a liver diet suggested by McCollum[8] and used by us in previous experimental work. This diet was devised as being adequate for nutrition. The addition of the ground liver made a high total protein content resulting in the passage of acid urine, with an average p_H of 6.2. We had previously found that the continued feeding of this diet over a long period of time resulted in an increase in blood pressure and the development of arteriosclerosis and chronic nephritis. This diet was

12. Nuzum, F. R.; Elliot, A. H.; Evans, R. D., and Priest, B. V.: The Occurrence and Nature of Spontaneous Arteriosclerosis and Nephritis in the Rabbit, Arch. Path. **10**:697 (Nov.) 1930.

given to ten of the animals already having spontaneous nephritis to see whether further elevation in blood pressure and further disturbance in kidney function would result.

Chemical and microscopic examinations of the urine were made at monthly intervals. The urine was obtained by pressure on the bladder in most instances, but occasionally animals were placed in metabolic cages and it was caught in containers.

At the end of two years, and again at the conclusion of the experiment one year later, the nonprotein nitrogen, the urea nitrogen, the carbon dioxide combining power of the blood and the phthalein excretion were determined.

Blood pressure determinations were made at monthly intervals. We have used the method of Van Eweyk [13] for the past eight years. Several thousand determinations have been made with it, most of them by one of us (Miss Priest). The technic of the procedure has been so developed that we are certain very satisfactory readings are obtained. Each reading is checked one or more times and the average taken. Various other methods for taking blood pressures in rabbits are described, but in our hands none has proved so satisfactory as this one.

URINARY CHANGES

At the beginning of the experiment (Oct. 1, 1925), the urine of each animal contained albumin and casts. Monthly examinations during the first year demonstrated the persistence of these abnormalities. All of the animals for months at a time had albumin and casts in their urine consistently. Occasionally the urine from one or two of them would be normal, but not longer than for two successive months.

During the second year (Jan. 1, 1927, to Jan. 1, 1928), the albumin increased, casts were more numerous, and normal urine was not obtained from the same animal for a period longer than one month.

Near the end of the third year twelve of the animals died, six in the alfalfa-barley group and six in the liver group, leaving four survivors in each group at the conclusion of the experiment on Jan. 1, 1929. At necropsy pneumonia was present in five animals, one had died from a ruptured blood vessel following the passage of a stomach tube, while in the remainder the cause of death was not apparent. Uremia might have been accountable, but uremic symptoms had not been present during life.

The exhibition of the liver diet during the year exaggerated in pronounced fashion the urinary abnormalities of the ten rabbits receiving it.

13. Van Eweyk, C., and Schmidtmann, M.: Zur Methodik der Blutdruckmessung beim Kaninchen, Virchows Arch. f. path Anat. **236**:420, 1921.

Casts and albumin were consistently present in greater amounts than in the urine from the remainder of the group.

That the renal changes were probably of a degenerative nature and not of inflammatory origin was evidenced by the fact that blood cells were not found in the urinary sediments.

There was a substantial increase of the nitrogenous bodies in the blood in every instance, except for rabbit 78, which showed a fall in the nonprotein nitrogen from 43.5 to 40 mg., and in the urea nitrogen from 29.4 to 20.8 mg. per hundred cubic centimeters (see the accompanying table). The kidneys of this animal were histologically unaltered, except for minor changes in the vessels and glomerular capsules. The phthalein

Blood Chemistry and Phthalein Excretion of Eight Surviving Rabbits *

	Carbon Dioxide Combining Power		Nonprotein Nitrogen		Urea Nitrogen		Phthalein (1 Hour)	
	Jan., 1928	Jan., 1929	Jan., 1928	Jan., 1929	Jan., 1928	Jan., 1929	Jan., 1928	Jan., 1929
Liver : rabbit 63	42.4	36.6	37.0	58.8	22.7	30.3	51	53
67	55.1	49.0	32.3	42.5	18.5	25.0	61	49
77	46.2	55.7	36.3	55.5	18.7	26.3	67	50
79	45.2	51.0	37.0	62.5	13.0	38.4	51	35
Barley : rabbit 61	32.8	30.0	40.0	58.8	22.7	31.2	74	85
62	51.9	55.7	36.4	47.6	21.4	23.8	66	46
69	51.9	50.0	32.3	32.2	12.5	17.6	46	39
78	55.7	61.4	43.5	40.0	29.4	20.8	83	70
Average (liver)	47.2	48.0	35.6	54.8	18.2	30.0	57.5	46.7
Average (barley)	48.0	49.2	38.0	44.6	21.5	23.3	67.2	60.0
Average entire group	47.6	48.6	36.8	49.7	19.8	26.6	62.3	53.3

* The first four rabbits in the table belong to the liver diet group, the second four to the group fed the alfalfa-barley mixture. The figures in the left-hand column under each heading represent the determinations made at the end of the two year period; the figures in the right-hand column, those made at the conclusion of the experiment one year later. At the bottom of the table are the average values for the two groups of four animals, and for the entire group of eight animals.

excretion was diminished in each instance, with likewise one exception. Rabbit 61 had an excretion of 74 per cent at the end of the two year period, increasing to 85 per cent at the conclusion of the experiment, but as the blood metabolites had increased during this time and chronic nephritis was present histologically, this may have been due to a technical error.

In comparing the group fed liver with that fed alfalfa-barley, it is seen that the changes were more pronounced in the former. The average value of the nonprotein nitrogen of these four animals had increased from 35.6 to 54.8 mg., while in the other group the increase was only from 38.0 to 44.6 mg. The urea nitrogen of the liver group increased from 18.2 to 30 mg., as compared with an increase from 21.5 to 23.3 mg. for the remaining animals. The decrease in the phthalein

excretion—from 57.5 to 46.7 per cent in the first group and from 67.2 to 60 per cent in the second—was also in accord.[14]

The changes in the carbon dioxide combining power were too slight to be of significance. They are tabulated as a matter of interest.

It would seem that spontaneous renal damage in the rabbit is accompanied by retention of nitrogenous substances in the blood, and by a decrease in the phthalein excretion, and that the feeding of a liver diet serves to enhance the damage already present, as measured clinically by these changes.

BLOOD PRESSURE

As mentioned, blood pressure determinations were made monthly on each animal, every reading being checked at least once. There was a gradual increase in pressure in all of the animals, except the controls. Wide variations were at times encountered from month to month (perhaps as a result of extraneous factors, such as season, temperature, time of day, restlessness during the determination, etc.), which are reflected on the charts, but the general trend was upward. The liver diet group showed a more rapid increase and a higher terminal average pressure.

Because of the wide fluctuations, it is perhaps inaccurate to compare actual values, but the figures may be of some interest. The animals on the liver diet, with an average initial pressure of 86 mm. of mercury, showed at the conclusion of the experiment one year later a pressure of 118 mm., or an increase of 32 mm. For the same period of time, the animals not receiving liver showed an increase from 98 to 112 mm., or only 14 mm.

It appears that the elevation of blood pressure in our animals was roughly proportional to the degree of the urinary changes, to the blood chemistry alterations and to the decrease in phthalein excretion. That renal (and arterial) injury is reflected to a certain extent in the height of the blood pressure in man seems fairly certain. Allbutt[15] has said that the pressure rises in every case of primary contracted kidney and in most cases of secondary contraction wherein the glomeruli are widely affected. By analogy, we might expect the same phenomenon in the rabbit, and our results indicate such a possibility. At any rate, the conclusion is justified that spontaneous nephritis in the rabbit, if of suffi-

14. It may be argued that the differences in these figures are not statistically significant, as the values do not lie well outside the standard deviation of the mean. Since, however, the other elements of the study tend in the same direction, namely, as indicating a more severe renal lesion in the liver diet group, the differences are suggestive.

15. Allbutt, T. C.: Arteriosclerosis, New York, The Macmillan Company, 1925, p. 13.

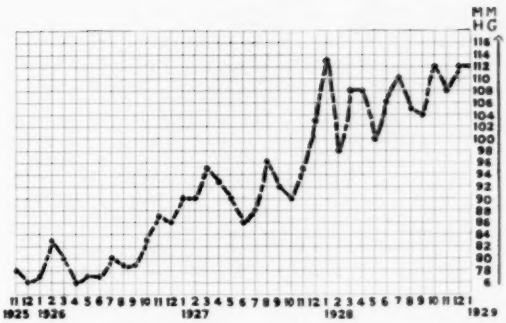

Chart 1.—The blood pressure range for all the animals for the entire thirty-six months, with the exception, during the last year, of those fed the liver diet. Each reading on the chart expresses the average of all determinations made at that particular time.

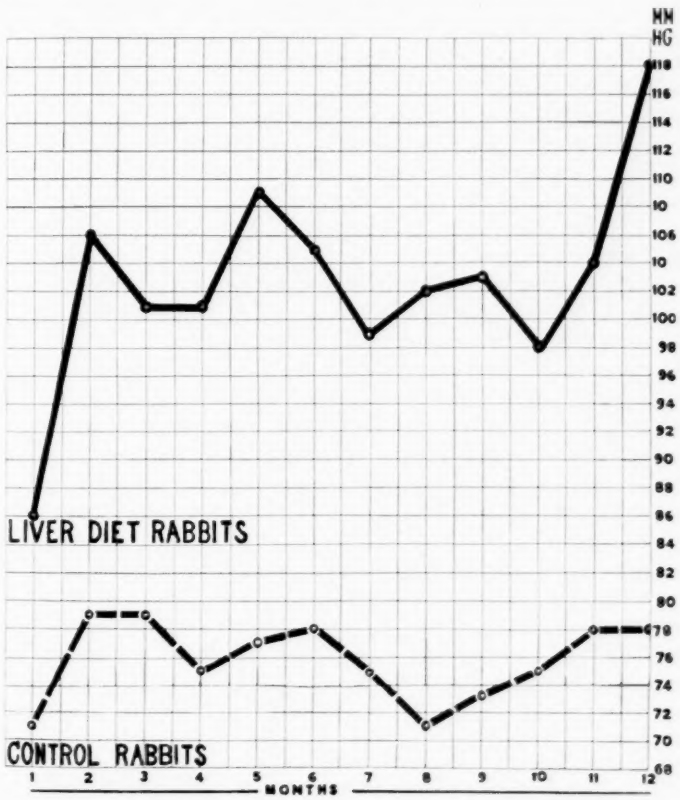

Chart 2.—Blood pressure range of ten rabbits with spontaneous nephritis fed a high liver diet for one year compared with that of twelve control animals.

cient degree to be recognized clinically, is accompanied by an elevation in blood pressure not encountered in normal rabbits. The feeding of a high protein (liver) diet causes a further increase in pressure.

SUMMARY

1. Spontaneous nephritis in the rabbit, pathologically of a diffuse type and indistinguishable from that experimentally produced, occurred in approximately 8 per cent of supposedly normal animals.

2. It was evidenced in the living animal by the persistent presence of albumin and casts in the urine, retention of nitrogen bodies in the blood, decrease in phthalein excretion and elevation of blood pressure. These manifestations were roughly proportional to one another and to the degree of renal injury.

3. The exhibition of a high protein (liver) diet, given over a period of one year to animals that for two years previously had had a well marked spontaneous chronic nephritis, exaggerated the foregoing manifestations.

4. Experimentally fed high protein diets increased preexistent renal damage.

DIAGNOSIS OF SYPHILITIC AORTITIS UNCOMPLICATED BY AORTIC REGURGITATION OR ANEURYSM

COMPARISON OF CLINICAL AND NECROPSY OBSERVATIONS IN ONE HUNDRED AND FIVE PATIENTS

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The early diagnosis of syphilitic aortitis, before the development of aortic regurgitation or aneurysm, is a question of fundamental importance in the treatment of cardiovascular syphilis. When the aortic valves have been so distorted as to produce incompetency or when the wall of the aorta has weakened to the point of producing saccular dilatation, the utmost to be expected of treatment is some degree of symptomatic relief and the possible prolongation of life to more than the usual span of about two years from the appearance of symptoms.[1] If, however, involvement of the aorta can be recognized before these anatomic disasters have occurred and if appropriate treatment is instituted, it is reasonable to hope for symptomatic relief in a higher proportion of cases and prolongation of useful life for a much longer period of time.

That the problem is deserving of careful study is indicated by the gradually increasing death rate from organic heart disease, by the actuarial estimates that 2 per cent of the total population of the United States are suffering from the conditions grouped under this classification and that the annual quota of deaths from this cause now approximates 200,000,[2] and from the frequency with which syphilis causes heart dis-

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1. Moore, J. E., and Danglade, J. H.: The Treatment of Cardiovascular Syphilis: A Study of the Duration of Life in Forty-One Treated and Untreated Patients with Regurgitation and Aneurysm, Am. Heart J. **6**:148, 1930.

2. Dublin, L. I.: Statistical Aspects of the Problem of Organic Heart Disease, Am. Heart J. **1**:359, 1926.

ease. In New York, an analysis of 1,001 cases of heart disease indicated that about one tenth of these cases were syphilitic.[3] In Baltimore, with a larger Negro population, Carter and Baker[4] have recently found that 15.1 per cent of 3,223 cases of heart disease in the wards of the Johns Hopkins Hospital were due to syphilis. With these estimates as a base line, it is easy to arrive at the conclusion that there must be, at any one time, about 240,000 persons in the United States with definite cardiovascular syphilis, of whom more than 20,000 die each year. In the light of the present state of diagnostic acumen, it would seem that the vast majority of these afflicted persons have already passed through the stage of simple aortitis to aortic insufficiency or aneurysm.

TABLE 1.—*The Incidence of Cardiovascular Syphilis Among Ten Thousand Syphilitic Patients (of Whom Six Thousand Four Hundred and Twenty had Late Syphilis [Turner])*

		Aortitis	Aortic Insufficiency	Angina Pectoris	Aneurysm	Other	Percentage of Total Late Cases with Cardiac Involvement		
White	Male	51	37	8	17	2	8.7	7.2	10.1
	Female	19	11	1	3	4	4.8		
Negro	Male	144	86	5	51	20	17.9	11.4	
	Female	118	40	3	18	11	7.2		
Percentage of late syphilis, showing various types of cardiovascular involvement		5.1	2.7	0.2	1.2	0.5			

ESTIMATES OF THE FREQUENCY OF SYPHILITIC AORTITIS

The frequency of aortic syphilis may also be estimated from the standpoint of its clinical incidence in a large population of syphilitic patients or of the frequency with which it is discovered at necropsy on syphilitic persons. Turner[5] has published data bearing on the former point, based on the admission diagnoses of 10,000 syphilitic patients in this clinic. His figures, reproduced in table 1, show the usual predominance of cardiovascular syphilis in Negroes as compared to white persons, and in males as opposed to females. They also indicate that in a syphilis clinic for ambulatory patients, the diagnosis of syphilitic aortitis, uncomplicated by aortic regurgitation or aneurysm, has been

3. Wyckoff, J., and Lingg, C.: Statistical Studies Bearing on Problems in the Classification of Heart Diseases: II. Etiology in Organic Heart Disease, Am. Heart J. **1**:446, 1926.

4. Carter, E. P., and Baker, B. M., Jr.: Certain Aspects of Syphilitic Cardiac Disease, Bull. Johns Hopkins Hosp. **48**:315 (May) 1931.

5. Turner, T. B.: The Race and Sex Distribution of the Lesions of Syphilis in Ten Thousand Cases, Bull. Johns Hopkins Hosp. **46**:159, 1930.

made as frequently as that of all other types of syphilitic heart disease. To what extent this diagnosis is justifiable is the part of the present and a subsequent study to discover.

As to the incidence of aortitis in syphilitic patients at necropsy, there are widely divergent estimates, apparently depending on the minuteness with which pathologic studies are made. In Germany, on the basis of 23,105 necropsies from the Rudolf Virchow Krankenhaus, Langer [6] maintained that from 70 to 80 per cent of all syphilitic patients show anatomic evidence of involvement of the aorta (simple aortitis, aortic regurgitation or aneurysm). We reproduce here two charts based on Langer's material, the first of which shows the incidence of syphilitic aortitis by years; the second, its incidence among syphilitic persons in

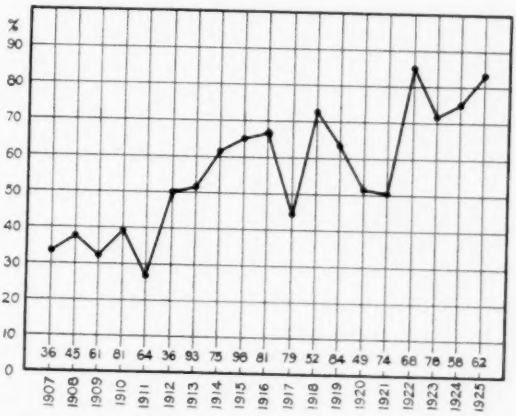

Chart 1.—Curve to show the percentage incidence of syphilitic aortitis in autopsies on syphilitic patients. The figures on the base line show the number of cases of syphilis found at necropsy. Based on 23,105 necropsies from the Rudolf Virchow Krankenhaus (Langer, E.: München. med. Wchnschr. **73**: 1782, 1926). Aortitis was diagnosed ante mortem in 284 cases, or 39.3 per cent; it was not diagnosed ante mortem in 437 cases, or 60.7 per cent.

different age groups. Langer pointed out that of the 721 cases of syphilitic aortitis found at necropsy, only 284, or 39.3 per cent, were diagnosed during life. The sharp increase in the incidence of aortitis beginning in 1912 and continuing thereafter is attributed by Langer (and by others in Germany, on the basis of similar studies) to the influence of the introduction of arsphenamine into the treatment of syphilis. It seems equally possible to explain it, however, on the basis of more

6. Langer, E.: Die Häufigkeit der luetischen Organveränderungen insbesondere der Aortitis luetica, München. med. Wchnschr. **73**:1782, 1926.

accurate pathologic study with increasing knowledge of the microscopic appearance of aortic syphilis.

In this country, Warthin has been constantly insistent on the importance of syphilis of the aorta. His most recent figures report [7] gross or microscopic evidence of syphilis (more usually the latter) in 90 per cent of 490 autopsies on syphilitic patients over 25 years of age. Incidentally, the percentage incidence of aortitis in syphilitic adults reported by him for the decade from 1909 to 1919 is 97.6, and for the decade from 1919 to 1929, 86.3, lending weight to the opinion that added pathologic experience and not the effect of arsphenamine is the factor responsible for the increased incidence in Langer's material.

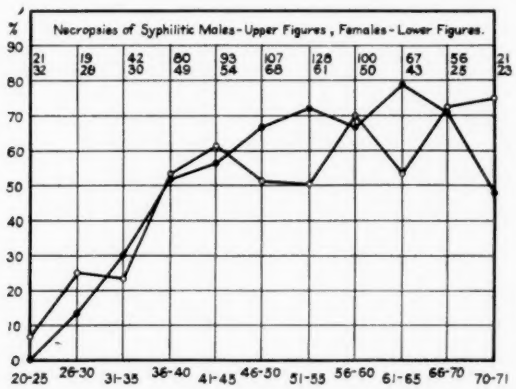

Chart 2.—Percentage incidence of syphilitic aortitis among syphilitic patients at different age groups—necropsy data from Langer.

It is probably true, therefore, that invasion of the aorta by the Treponema is as universal in syphilitic infection as is invasion of the nervous system. Every syphilitic patient is a potential candidate for the development of cardiovascular syphilis. It is necessary to distinguish, however, between invasion and involvement of the aorta, in the same sense as we have come to distinguish between invasion of the nervous system and tissue involvement extensive enough to produce the clinical picture of neurosyphilis. One can hardly expect of any clinician sufficient expertness in diagnosis to enable him to detect the presence of a single microscopic focus of round and plasma cell infiltration in the aortic wall. Unfortunately, Warthin, who has done the most careful work in the pathology of syphilis, has published no data indicating the

7. Warthin, A. S.: The Lesions of Latent Syphilis, South. M. J. **24**:273, 1931.

proportion of cases in which the pathologic lesions were so insignificant as to be incapable of producing symptoms or physical signs, as compared with those in which the lesion was so extensive as to have resulted in actual clinical disease. Adequate studies from other pathologists are also lacking, since few have devoted as much attention as Warthin to the minutiae of microscopic pathologic diagnosis in syphilis.

From the clinical standpoint, there are wide differences of opinion as to the possibility of arriving at an accurate diagnosis of syphilitic aortitis in the absence of aortic regurgitation or aneurysm. At the present time, the consensus among internists probably favors the group which holds that, in view of the frequency of arteriosclerotic changes in the aortas of many persons, syphilitic or otherwise, after the age of 50, such a diagnosis cannot be safely made.[8] This opinion is, of course, based on the fact that syphilitic aortitis is often found at necropsy when unsuspected during life, and conversely that it has sometimes been absent at necropsy when all the so-called classic symptoms and signs, both physical and roentgenologic, were present during life.

THE POSSIBILITY OF CLINICAL DIAGNOSIS OF UNCOMPLICATED AORTITIS

It occurred to us that information of value as to the possibility of accurate clinical diagnosis of uncomplicated aortitis might be obtained by an approach from two angles: (1) a comparison of the clinical with the necropsy observations in those patients in whom syphilitic aortitis was discovered at necropsy, whether or not recognized in life, and (2) a survey of the clinical outcome, for evidence of progression to valvular incompetency or aneurysm or, when possible, to the autopsy table, in a series of patients in whom the diagnosis was made during life. The present article describes the results of a study of the first group of patients.

The records of the department of pathology of the Johns Hopkins Hospital from 1910 to 1930 were reviewed. All cases with aortic regurgitation or aortic aneurysm were excluded from consideration, as were likewise certain instances in which the presence of extensive aortic arteriosclerosis rendered doubtful the pathologic diagnosis. After these exclusions, there remained 105 cases with definite uncomplicated syphilitic aortitis, in all of which the pathologic diagnosis was made on the basis of grossly visible lesions of syphilis in the aorta, confirmed in 84 by microscopic examination. The clinical records of these patients were then studied, in order to determine the frequency with which the diagnosis was accurately made during life.

8. Conner, L. A., in discussion on Steel, D.: Am. Heart J. **6**:65, 1930.

Before proceeding to a discussion of the results it is necessary to consider the obvious criticism that the pathologic diagnosis of syphilitic aortitis was sometimes or often erroneous, and that therefore conclusions drawn from such material are untrustworthy. To this, one can reply only that the gross and histologic tissue examinations were made over a twenty year period by many members of the staff of the department of pathology, and that in most instances both gross and microscopic specimens were also reviewed by a senior member of the staff. Furthermore, the pathologic picture of syphilitic aortitis is quite characteristic, and, except in the presence of extensive aortic atherosclerosis, is not easily confused with other changes. In most of the instances here reported there was confirmatory clinical or pathologic evidence of syphilis. Unfortunately, specimens have not been preserved as a routine measure, so that we were unable to examine personally the aortas in question.

TABLE 2.—*A Comparsion of the Correctness of the Clinical and Pathologic Diagnosis of Syphilitic Aortitis in One Hundred and Five Cases so Diagnosed at Necropsy*

		Syphilitic Aortitis		Diagnosis Could Have Been Suspected on Basis of			Diagnosis Obscured by Other Cardiovascular Lesions	Heart and Aorta Thought Clinically Normal	Patient Too Ill for Adequate Examination
	Total Cases	Definitely Diagnosed	Suspected	Physical Signs	Symptoms	Both			
Medical service	63	4	12	12	2	9	9	11	4
Surgical service	43	..	1	5	4	3	3	23	3
Total	105	4	13	17	6	12	12	34	7
Percentage of total		16.2			33.3		11.4	32.3	

The 105 patients in this series entered the hospital for a wide variety of causes. Their histories were taken and the first physical examination made by an intern; in most instances, the physical examination was repeated once or several times by senior members of the resident staff and by visiting consultants. Naturally, the twenty year period covered by the study necessitates the use of records made by many physicians of varying degrees of clinical proficiency and with greater or less special interest in cardiovascular disease or in syphilis. In many instances, the acuteness of the final illness prevented careful history taking, or its character prevented accurate physical examination of the heart and the aorta. Many records, therefore, fail to include either positive or negative statements as to the presence or absence of suggestive signs or symptoms. In such cases, we have recorded "no data available" and have attempted to keep these limitations in mind throughout the study. In one sense, however, this is less of a drawback for our purposes than might be anticipated. We may fairly consider these clinical records to represent the average of intelligent medical practice of the past twenty years.

The results of this survey are summarized in table 2. Sixty-three patients died in the medical wards of the hospital; 42 were in such various surgical services as obstetrics, gynecology, urology and general surgery. The diagnosis of syphilitic aortitis was correctly made before death in 4 instances, or 3.8 per cent. In an additional 13 patients, one or more of the several examiners had suspected that there was something wrong with the patient's aorta, and had recorded an impression diagnosis of dilated aortic arch, aneurysm or aortic regurgitation, but in none of these instances was the impression taken sufficiently seriously as to be included in the final diagnosis. However, including these 13 patients as clinical successes, the diagnosis was correctly made or suspected in only 16.2 per cent of the group. All but 1 case were recognized on the medical service, and even in the one exception, the suspicion of syphilitic aortitis was raised by a consulting internist.

In all the cases correctly diagnosed or suspected, either the usual symptoms of substernal pain, dyspnea on exertion or paroxysmal nocturnal dyspnea or the classic physical signs of widened retromanubrial dulness, pulsation in the suprasternal notch, an aortic systolic murmur or an altered tympanitic bell-like quality of the aortic second sound, or both symptoms and signs together, were present. These symptoms and signs, however, were also present to some degree in an additional 35 patients, or 33.3 per cent of the series, in whom their significance was completely disregarded by all examiners. In these patients the question of syphilitic involvement of the aorta was not mentioned in impression or in final diagnosis, though it is certainly fair to say that a higher index of suspicion on the part of the various examiners and consultants should have prompted some consideration of the possibility. In 17 of these patients, physical signs were present without symptoms; in 6 instances, suggestive symptoms (usually antedating the symptoms of the final illness) occurred in the absence of physical signs; and in 12 patients, even the association of signs and symptoms was overlooked.

In the remaining 53 patients, the diagnosis of syphilitic aortitis apparently could not have been made. Seven of these were moribund on admission to the hospital, and there was no opportunity for careful history taking or examination of the heart. In 12, the symptoms and signs of aortitis were obscured by some other cardiovascular condition such as hypertensive cardiorenal disease, rheumatic heart disease with auricular fibrillation, bacterial endocarditis, adherent pericardium, etc. Thirty-four patients, 11 medical and 23 surgical, died with no suspicion in the mind of any examiner that there was anything at all the matter with the heart or aorta. Whether this is because all suggestive symptoms and signs were actually absent or because their presence was overlooked by inadequate or careless history taking or physical examination cannot be determined.

It is true, however, that the majority of these patients were admitted to the hospital for an acute illness unrelated to syphilis or heart disease; that attention was thus directed away from the heart, and that in many patients on the surgical services examination was not made by an experienced internist. It will presently be shown that, judging by the extent of the pathologic change found at necropsy, clinical diagnosis was not necessarily impossible in this last group of patients. Even assuming that it was impossible, we arrive, however, at the startling conclusion that of the total number of 105 patients, a correct antemortem diagnosis was made in 4 and suspected in 13, while it might have been made in 52, had the signs and symptoms actually present been correctly interpreted.

TABLE 3.—*Race and Sex Distribution and Age at Death of One Hundred and Five Patients with Syphilitic Aortitis*

		Total Cases	Age at Death				
			21-30	31-40	41-50	51-60	61+
White	Male	26	..	8	9	4	5
	Female	5	2	..	1	2	..
Negro	Male	53	5	12	24	6	6
	Female	19	2	12	3	..	2
Total		9	32	37	12	13	

TABLE 4.—*The Correctness of the Clinical Diagnosis of Syphilitic Aortitis as Influenced by the Cause of the Fatal Illness*

	Total Cases	Diagnosis Correctly Made or Suspected	Diagnosis Might Have Been Made	Diagnosis Obscured by Other Cardiac Findings	Diagnosis Missed (Impossible to Make?)
Fatal illness related to syphilis	24	6 (25%)	7 (29%)	5 (20%)	6 (24%)
Fatal illness unrelated to syphilis which, if diagnosed, was an incidental finding	81	10 (12%)	29 (37%)	7 (8%)	35 (43%)

The race and sex distribution and the age at death of this group of patients are shown in table 3. The usual preponderance of Negroes and of males is obvious. The fact that death occurred before the age of 51 in 75 per cent of the group is a partial answer to the question of the difficulty of differential diagnosis between syphilitic aortitis and aortic arteriosclerosis. Atherosclerosis of the aorta in these younger patients was rare, and, when present, was not often extensive.

The fatal illness that brought the patients to the hospital was in some way related to syphilis in 24 instances. These were patients with, for example, tabes dorsalis and infected urinary tracts, arsphenamine poisoning, syphilis of the liver, cardiac failure secondary to syphilitic aortitis, etc. In 81 patients, the final illness had nothing to do with syphilis, which, if diagnosed, was entirely an incidental finding. Here

the clinical diagnoses and the primary causes of death include such widely divergent conditions as lobar pneumonia, typhoid fever, septicemia, carcinoma, postoperative peritonitis or ileus, strangulated hernia, etc. That a high index of suspicion aids in the recognition of syphilitic aortitis is indicated by the relative success of clinical diagnosis in the two groups. In only 6, or 24 per cent, of the patients whose illness was directly related to syphilis were the heart and aorta regarded as normal, while this situation existed in 35, or 43 per cent, of those in whom syphilis was only a relatively unimportant incident of the fatal illness.

From the clinical standpoint, a most difficult differential diagnostic decision is between syphilitic aortitis and hypertension. Both may produce substernal discomfort, dyspnea on exertion, widening of the aorta and a change in the intensity of the aortic second sound. Paroxysmal nocturnal dyspnea is, however, a frequent feature of syphilitic aortitis, especially with aortic regurgitation, but is uncommon in hypertension

TABLE 5.—*The Correctness of the Clinical Diagnosis of Syphilitic Aortitis as Influenced by the Presence or Absence of Hypertension*

	Total Cases	Diagnosis Made or Suspected	Diagnosis Could Have Been Made	Diagnosis Obscured by Other Cardiac Lesions	Diagnosis Missed (Impossible to Make)
Hypertension present	30	8 (26%)	10 (33%)	4 (13%)	8 (26%)
Hypertension absent	75	9 (12%)	25 (33%)	9 (12%)	32 (42%)

without left ventricular failure; many experienced examiners believe that while accentuation of the aortic second sound is common to the two groups, a tympanitic bell-like alternation of its quality is peculiar to and characteristic of syphilitic aortitis (Carter and Baker [4]). Analysis of this material indicates, as has already been repeatedly pointed out, that hypertension is not a frequent accompaniment of syphilitic aortitis, and that when present it usually depends on some other definite cause, such as chronic nephritis or arteriolar sclerosis. Since all patients with aortic regurgitation have been excluded from this study, systolic elevation of the blood pressure to 150 mm. or over has been taken as a criterion of arterial hypertension. On this basis the blood pressure was normal in 75 patients; the systolic blood pressure ranged between 150 and 200 in 23, and was over 200 in 7. Curiously enough, in spite of the confusion with which every clinician is faced in the individual case, the diagnosis of syphilitic aortitis was correctly made more often in the group with than in that without hypertension, and it was completely overlooked, the heart and aorta being regarded as normal, much less frequently than when hypertension was absent. Perhaps this is because the existence of arterial hypertension focused attention on

the cardiovascular apparatus which otherwise was devoted to clinical abnormalities unrelated to syphilis or hypertension. At any rate, the clinician's diagnostic difficulties were actually less, instead of greater, if the aortitis was complicated by elevation of the blood pressure.

The association of other clinical or pathologic evidence of syphilis with aortitis was of incidental interest to this study. A Wassermann test of the blood was done in 81 of the 105 patients, and was found to be positive in 61, or 75.3 per cent, and negative in 20, or 24.7 per cent. Of the 20 patients with negative Wassermann reactions of the blood, 2 showed some other clinical evidence of syphilis, and 3 more gave a definite history of infection. In 14 of the 20, there was nothing whatever to suggest syphilis either clinically or pathologically except the syphilitic aortitis, though in no instance was the pathologic study (especially microscopic) so detailed as that carried out by Warthin. From the purely pathologic standpoint, aortitis with its associated secondary cardiac changes of hypertrophy, chronic fibrous myocarditis, etc., was

TABLE 6.—*The Correctness of the Clinical Diagnosis of Syphilitic Aortitis as Influenced by the Extent of Pathologic Damage*

Degree of Pathologic Change	Total Cases	Diagnosis Correctly Made or Suggested	Diagnosis Might Have Been Made	Diagnosis Obscured by Other Cardiac Findings	Diagnosis Missed (Impossible to Make?)
Slight	23	3 (13%)	10 (43%)	.....	10 (43%)
Moderate	29	4 (14%)	9 (31%)	4 (14%)	12 (41%)
Extensive	49	10 (20%)	16 (32%)	8 (16%)	15 (30%)

the only anatomic evidence of syphilis in 61 (nervous system usually not examined at necropsy), while in 44 there was associated pathologic evidence of syphilis, involving the liver in 14 patients, the central nervous system in 15, the testis in 7 and other organs in 11.

An attempt was made to estimate the degree of syphilitic involvement of the aorta from the pathologic standpoint, and to compare this with the accuracy of clinical diagnosis. While this was not always possible, because of inadequate pathologic description, nevertheless, a division into three groups, on the basis of slight, moderate and extensive involvement, was attempted. Slight involvement includes those cases in which there were a few or many discrete patches of syphilitic inflammatory tissue in the aortic wall without much loss of elasticity. Extensive involvement indicates widespread, confluent infiltration with or without intimal ulceration and with marked loss of elasticity and wide dilatation (not aneurysmal) of the aortic wall. Moderate involvement includes cases intermediate between these two. As one might expect, clinical accuracy in diagnosis increases in proportion to the degree of pathologic change present. It is surprising to note, however, that symptoms, signs

or both, which made the clinical diagnosis permissible, were present in 13 of 23 patients with slight pathologic involvement (56 per cent), while these same clinical abnormalities were noted to be present in only 26, or 52 per cent, of those with extensive pathologic changes. The heart and aorta were regarded as clinically normal in 30 per cent of those with extensive aortitis. In some cases, at least, this must have depended on inaccurate clinical observation, as in the patient whose pathologic note read "huge fusiform dilation of the aorta," but in whom nothing abnormal was found in the heart or aorta before death.

It seems to us interesting and important that the x-ray was so infrequently used as a diagnostic aid in this group of patients. In only 29 patients in the entire group was a roentgenographic examination carried out. In 25 of these there was definite roentgen evidence of dilatation of the aorta. We have personally examined these plates; in some instances our interpretation had been confirmed earlier by fluoroscopy carried out by a roentgenologist. There have been numerous studies made on the roentgenologic diagnosis of syphilitic aortitis, one of the most recent of which (Steel[9]) concluded that the lesion can be suspected when: (1) a diffuse dilatation is present and is associated with a normal heart silhouette, or (2) a dense, high aorta is present in a young person without previous hypertension. Obviously, teleroentgenographic and fluoroscopic examinations should be made in every syphilitic patient with symptoms or signs suggesting aortic involvement, and many cases of probable aortitis without symptoms or definite physical signs would be detected if this examination were routine in every patient with late syphilis.

THE CRITERIA OF DIAGNOSIS OF UNCOMPLICATED SYPHILITIC AORTITIS

Carter and Baker,[4] while they did not emphasize the question of uncomplicated syphilitic aortitis, expressed the belief that the diagnosis of cardiovascular syphilis is permissible if any five of the following seven criteria are clinically demonstrable:

1. The history of a relatively abrupt and unexpected onset of the symptoms of circulatory embarrassment.
2. The presence of a positive Wassermann reaction.
3. A demonstrable increase in the retromanubrial dulness in the second interspace, and a change in the tonal quality of the aortic second sound. The fluoroscopic evidence of aortic dilatation.
4. An absence of the signs of mitral disease, connoting rheumatic infection.
5. The history of paroxysmal dyspnea, often nocturnal.
6. The history of pain, particularly paroxysmal pain.
7. Progressive cardiac failure.

9. Steel, D.: The Roentgenographic Diagnosis of Syphilitic Aortitis in a Review of Forty Proved Cases, Am. Heart J. **6**:59, 1930.

We have analyzed our 105 cases for the incidence of these various signs and symptoms, with the result shown in table 7. These data indicate that the most consistently positive finding is roentgen evidence of aortic dilatation, and this even without the aid of fluoroscopy, from which additional information of value as to the elasticity of the aortic wall may be gathered. Next in importance is a history of symptoms of circulatory embarrassment, usually dyspnea on exertion, which in this series was more often insidious than abrupt in onset. Increased retrosternal dulness occurred in almost half the patients; one suspects from the roentgen evidence that more careful physical examination would have shown its presence in a still higher proportion. The presence of a tympanitic accentuated second aortic sound was noted in only 25.8 per cent, though here, too, one is relatively certain that had a single experienced examiner been able to see all these patients, the incidence of this abnormality would have been much greater. The interpretation of this

TABLE 7.—*The Incidence of Various Symptoms and Signs in Patients with Syphilitic Aortitis Proved at Necropsy*

	Total Cases with Information Available	Number of Patients Showing Symptom or Sign	Percentage
History of circulatory embarrassment.	88	44	50.0
Widened retrosternal dulness.....	89	44	49.4
Accentuated tympanitic A_2.....	89	23	25.8
Roentgen evidence of widened aorta...	29	25	86.0
Paroxysmal dyspnea	90	7	7.7
Pain	89	16	17.9
Cardiac failure (not terminal).....	92	24	26.0

change in quality of the heart depends on experience. Paroxysmal dyspnea and substernal pain or discomfort were much less frequent than we had anticipated.[10]

Twenty-two patients in this series showed none of the symptoms or physical signs named. Thirty-three more had one or two symptoms, signs or both, while in the remainder in whom the data were available, from three to six of the criteria listed were present (not including Carter and Baker's second and fourth criteria, namely, the presence of a positive Wassermann reaction of the blood and the absence of mitral disease).

On this basis, we should tabulate, in order of relative importance, the following diagnostic criteria of uncomplicated syphilitic aortitis:

1. Teleroentgenographic and fluoroscopic evidence of aortic dilatation.
2. Increased retromanubrial dulness.
3. A history of circulatory embarrassment.

10. Additional suggestive physical evidences of syphilitic aortitis are: (1) a visible and palpable pulsation in the suprasternal notch in the absence of hypertension, and (2) a rough systolic murmur loudest at the aortic area but often also audible at the apex.

4. A tympanitic, bell-like, tambour accentuation of the aortic second sound.
5. Progressive cardiac failure.
6. Substernal pain.
7. Paroxysmal dyspnea.

We believe that in a patient with known late syphilis (whether or not the Wassermann reaction of the blood is positive), the presence of any three of these criteria is strong evidence for the diagnosis of syphilitic aortitis, while the presence of any two of them renders the diagnosis probable. In a patient with a negative Wassermann reaction of the blood, with no history or other physical evidence of syphilis and without obvious disease of the mitral valve, the presence of any four of these symptoms or signs is also clinical justification for the diagnosis.

This position will, we hope, be materially strengthened by the corollary of the present study. In contrast to following, as we have done, the patient with syphilitic aortitis backward from the necropsy table through his clinical course, we must next follow a group of patients in whom the diagnosis was actually made during life to the necropsy table when possible, to see to what extent our clinical assumptions are justified. Such a study will form the subject of a subsequent paper of this series.

We believe, on the basis of this study, that it is possible to make the diagnosis of syphilitic aortitis in the absence of aortic insufficiency or aneurysm. The percentage of correct diagnoses will increase if the physician remembers that aortitis is a frequent pathologic finding in late syphilis, if he makes specific inquiry for possible symptoms, if he looks for the common physical signs, and, above all, if he uses teleroentgenologic examination as a routine measure in patients who have had syphilis for ten or more years.

We think that in the light of present knowledge it is fair to take the attitude that it is better for the patient, so far as the management and treatment of his syphilitic infection are concerned, to diagnose syphilitic aortitis on the basis of relatively slight suspicion during life, even if the lesion is not found at autopsy, than to fail to recognize it during life only to have it discovered at autopsy. Under the former circumstances, appropriate treatment and years of subsequent observation may well prevent the appearance of aortic insufficiency or aneurysm and preserve the patient for a long and useful life. Under the latter, we shall be too often chagrined by the pathologist's announcement that the patient died of syphilitic heart disease unsuspected clinically or of the unfortunate effects of too strenuous treatment which would not have been given had the true situation been known.

SUMMARY AND CONCLUSIONS

1. The early diagnosis of uncomplicated syphilitic aortitis is of fundamental importance for the treatment of cardiovascular syphilis.

2. The literature indicates that deaths from organic heart disease are increasing and that syphilis is responsible for from 10 to 15 per cent of all cases grouped under this heading.

3. In this clinic about 10 per cent of all patients with late syphilis show clinical evidence of cardiovascular syphilis.

4. Necropsy evidence, from this country and abroad, indicates that from 80 to 90 per cent of all adults with late syphilis show pathologic evidence of syphilis of the aorta.

5. In the 20 year period from 1910 to 1930, there came to necropsy in the Johns Hopkins Hospital 105 patients with uncomplicated syphilitic aortitis (without aortic regurgitation or aneurysm).

6. Sixty-three of these patients died while in the medical service; 42 were in various surgical services.

7. The clinical diagnosis of syphilitic aortitis was correctly made during life in 4 of the 105 patients; in 13 more, it was suspected that something was wrong with the aorta.

8. On the basis of the symptoms and physical signs recorded, the diagnosis might have been correctly made in 35 additional patients.

9. In 12 instances, the diagnosis was obscured by some other form of cardiovascular disease.

10. Thirty-four patients died with hearts and aortas thought clinically to be normal.

11. Syphilis was infrequently a major feature of the fatal illness. When it was, aortitis was more often diagnosed correctly than when the final illness was unrelated to syphilis.

12. Hypertension was an infrequent accompaniment of syphilitic aortitis, and in spite of the confusion it created in the individual case, its presence did not prevent correct diagnoses.

13. The Wassermann reaction of the blood was positive in 75 per cent of these cases, and negative in 25 per cent.

14. The success or failure of clinical diagnosis is compared with the extent of pathologic change present in the aortas. Symptoms and signs permitting a diagnosis were present in about half of those who showed only relatively slight gross pathologic change.

15. The criteria of diagnosis are discussed, and seven frequently found symptoms or signs listed. Of these the most important are roentgenologic evidence of aortic dilatation, increased retrosternal dulness, a history of circulatory embarrassment and a change in the tonal quality of the aortic second sound.

16. In patients with proved late syphilis the presence of any three of the seven criteria listed is considered justification for the diagnosis of uncomplicated syphilitic aortitis.

EXPERIMENTAL RENAL INSUFFICIENCY PRODUCED BY PARTIAL NEPHRECTOMY

I. CONTROL DIET

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A review of the literature of experimental nephritis shows that investigators have failed to produce chronic renal lesions in experimental animals comparable to those noted in man. Chemicals and toxins may cause an acute fatal condition or one from which the animal will recover, but the most promising procedure seems to lie in the removal or destruction of renal tissue.

The literature dealing with the effects of experimental renal insufficiency induced by the operative removal of kidney tissue contains conflicting conclusions and results. The reasons for many of the differences can be accounted for by the lack of controlled experimental conditions, involving the dietary regimen, the amount of kidney tissue removed, the period of experimentation and the type of animal used. Since the efforts of many investigators to produce consistently such signs as hypertension, cardiac hypertrophy, polyuria, albuminuria, etc., have been unsuccessful or only partially successful, a series of experiments was devised to study certain phases of the problem on a large number of animals.

The first work on partial nephrectomy was done by Tuffier.[1] This investigator removed first one kidney and later a portion of the other. He concluded that there were no changes in the elimination of urine or urea.

After the removal of three fourths of the kidney tissue in dogs, Bradford [2] noted that death occurred in from one to six weeks from asthenia with great wasting. If approximately two thirds of the total

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1. Tuffier, T.: Études expérimentelles sur la chirurgie du reins, Paris, G. Steinheil, 1889; quoted by Pearce, R. M.: J. Exper. Med. **10**:632, 1908.

2. Bradford, J. R.: The Results Following Partial Nephrectomy and the Influence of the Kidney Upon Metabolism, J. Physiol. **23**:415, 1899.

kidney tissue was removed, a considerable and practically permanent increase in the urinary volume was observed, but there was no appreciable change in the amount of urea excreted. The removal of three fourths of the kidney tissue was followed by a marked increase in the urinary volume accompanied by an increase in the amount of urea excreted. Under the latter circumstances, there is an increase in the nitrogenous compounds in the blood and in the tissues, particularly the muscles. No evidence of cardiac hypertrophy or of arterial change was found. Bradford suggested that the tissues of the body and particularly the muscles broke down when the kidney tissue was markedly reduced. He stated that he had no evidence to determine whether this was due to the lack of an internal secretion of the kidney. No albuminuria was noted in these experiments.

In 1905, Pässler and Heinecke [3] presented the first evidence of hypertension associated with cardiac hypertrophy as a result of partial nephrectomy. These investigators removed one and one-half kidneys, and at frequent intervals removed small portions of the remaining hypertrophied tissue. Of the eighteen dogs operated on, seven lived at least four weeks without cachexia after the last operation and showed the signs of renal insufficiency. Of these animals, five showed definite cardiac hypertrophy, one was close to the upper limit of normal, and one was normal. The cardiac hypertrophy was judged by comparing the weight of the left with that of the right ventricle. In the seven dogs mentioned, the ratio was 2.26:1 as compared to the normal of 1.76:1. The hypertrophy was noted only in dogs that had renal insufficiency of high degree but did not develop cachexia. The blood pressure determinations were made by cannulating the femoral artery. The increase noted varied from 15 to 29 mm. of mercury with an average of 21.5 mm. Polyuria accompanied by albuminuria and cylindruria was obtained in these animals, but no edema was noted. It is of importance to emphasize that the hypertension persisted long after the necrotic stumps had been absorbed.

Bainbridge and Beddard [4] concluded, from their studies on cats, that the removal of three fourths of the kidney substance caused loss of appetite, wasting and death in a few days or weeks. There was an increase in nitrogen output only after the animal had lost 22 per cent or more of its body weight, which was similar to the results obtained with starving animals. The possibility of an internal secretion of the

3. Pässler and Heinecke: Versuche zur Pathologie des Morbus brightii. Verhandl. d. deutsch. path. Gesellsch. **9**:99, 1905.

4. Bainbridge, F. A., and Beddard, A. P.: The Relation of the Kidneys to Metabolism, Proc. Roy. Soc. **79B**:75, 1907.

kidney having any direct effect on nitrogen metabolism was excluded. They also found that there was not necessarily an increase in the volume of urine.

The work of Bainbridge and Beddard [4] was confirmed by Pearce [5] during the following year. No polyuria or constant albuminuria was noted.

Janeway [6] used Carrel's [7] method of reducing the kidney substance by ligating several branches of the renal artery. Two dogs lived for one hundred and four and one hundred and sixty-three days, respectively, and showed a moderate sustained hypertension (from 20 to 35 mm. of mercury above normal) with polyuria, albuminuria and cylindruria. It was noted that the hypertension was sustained after the infarcted kidney tissue had been absorbed.

Pilcher [8] ligated branches of the renal arteries of one dog and one cat. He made no note of the blood nitrogen level or of the blood pressure. He concluded that "with but one-fourth of the kidney substance functioning, the quantity of urine was practically normal; the urine contained no albumin or casts; cardiac hypertrophy did not occur."

Cash [9] reduced the renal tissue of dogs by excision and by ligation of the renal vessels. A moderate increase in blood pressure was obtained shortly after the operation, provided that the portion of the kidney that was deprived of its circulation was allowed to remain in situ. This increased blood pressure lasted but a short time and tended to return to normal. There was no appreciable change in the blood nonprotein nitrogen or total chlorides after a reduction of from 25 to 85 per cent of the kidney tissue. No edema was noted.

Rabbits were utilized by Anderson [10] in a study of renal insufficiency. He found that the operative removal of 70 per cent of the kidney tissue in rabbits does not produce hypertension even when prolonged renal insufficiency results. An increase was noted in the blood urea, which

5. Pearce, R. M.: The Influence of Kidney Substance Upon Nitrogenous Metabolism, J. Exper. Med. **10**:632, 1908.

6. Janeway, T. C.: Note on the Blood Pressure Changes Following Reduction of the Renal Arterial Circulation, Proc. Soc. Exper. Biol. & Med. **6**:109, 1909.

7. Carrel, A.: Note on the Production of Kidney Insufficiency by Reduction of the Arterial Circulation of the Kidney, Proc. Soc. Exper. Biol. & Med. **6**:107, 1909.

8. Pilcher, J. B.: On the Excretion of Nitrogen Subsequent to the Ligation of Successive Branches of the Renal Arteries, J. Biol. Chem. **14**:389, 1913.

9. Cash, J. R.: A Preliminary Study of the Blood Pressure Following Reduction of Renal Substance, with a Note on Simultaneous Changes in Blood Volume and Chemistry, Bull. Johns Hopkins Hosp. **35**:168, 1924.

10. Anderson, H.: The Relation of Blood Pressure to the Amount of Renal Tissue, J. Exper. Med. **39**:707, 1924: Experimental Renal Insufficiency, Arch. Int. Med. **37**:297 (March) 1926.

was initially greater in those that died early and was progressively increased in animals living for comparatively long periods. The specific gravity of the urine of these rabbits tended strongly toward fixation. Albuminuria and cylindruria were absent.

Allen, Scharf and Lundin [11] have reviewed their work on partially nephrectomized dogs, sheep and goats. These investigators found that dogs will die if more than 75 per cent of the kidney tissue is removed, but goats will survive in apparently good health with but one fifth of one kidney. In dogs on a normal diet, elevations in blood pressure of from 20 to 30 mm. were obtained. These animals showed a retention of nitrogenous materials and an impaired water excretion, large volumes of administered water being retained in the blood and tissues while the urine output was scanty and the specific gravity relatively high. Marked polyuria was obtained in sheep and goats. A moderate anemia developed in these animals.

Mark [12] succeeded in producing a noticeable renal insufficiency in five dogs by ligation of the branches of the renal arteries. These animals continued in good health with no marked symptoms or loss of weight. His findings with the animals on a control diet were as follows: There was no polyuria; there was a definite loss of ability to concentrate the urine but no loss of ability to dilute it; there was a loss of ability to excrete definite amounts of ingested urea within twenty-four hours, whereas the normal dog could excrete the same amount within from two to three hours; the blood pressure remained normal.

In 1930, Mark and Geisendörfer [12a] produced a renal insufficiency in dogs by ligation of the renal arteries and subsequent removal of the remaining kidney. They demonstrated a definite hypertension and cardiac hypertrophy in five dogs that survived the operations for an appreciable length of time. The cardiac hypertrophy was judged by microscopic measurements of the diameters of the heart fibers as well as by gross examination.

Verney,[13] working with a perfused heart-lung double-kidney preparation, obtained an immediate increase in urinary flow after the ligation of a branch of the renal artery. The reduced kidney tissue responded

11. Allen, F. M.; Scharf, R., and Lundin, H.: Clinical and Experimental Renal Deficiency, J. A. M. A. **85**:1698 (Nov. 28) 1925.

12. Mark, R. E.: Untersuchungen über die Nierenfunktion: Ergebnisse partieller Nierenarterienunterbindung am Hunde, Ztschr. f. exper. Med. **59**:601, 1928.

12a. Mark, R. E., and Geisendörfer, H.: Untersuchungen über die Nierenfunction: Zur Frage des Zusammenhanges von Nierenmasse, Herzhypertrophie und Blutdrucksteigerung, Ztschr. f. d. ges. exper. Med. **74**:350, 1930.

13. Verney, E. B.: Experimental Reduction of Renal Tissue, Lancet **1**:645, 1929.

at a constant blood pressure as though it was subjected to an increased perfusion pressure. In other words, "the pressure stimulus to secretion has become more effective."

Ferris and Hynes [14] removed one kidney and ligated the renal arteries of the other. A marked rise in blood pressure occurred shortly after the operation. This elevation was followed by a gradual fall ending with a base line from 10 to 15 mm. above normal.

In a preliminary report dealing with chronic renal insufficiency in dogs, Apfelbach and Jensen [15] reported the results obtained after injecting particles of charcoal into the renal artery. They listed the following changes: a decrease in the ability of the kidney to concentrate and dilute urine, nitrogen retention in the blood, polyuria, a decrease in body weight and a decrease in the carbon dioxide combining power; edema and arterial hypertension did not occur. They stated that death could not be ascribed to renal insufficiency unless the foregoing syndrome occurred.

A brief summary of the results obtained after partial nephrectomy in various species will emphasize the conflicting views concerning the experimental production of arterial hypertension, renal insufficiency and cardiac hypertrophy:

1. Arterial hypertension was noted in the chronic state of renal insufficiency by Pässler and Heinecke,[3] Janeway,[6] Allen, Scharf and Lundin [11] and Mark and Geisendörfer; [12a] hypertension in the acute stage by Cash [9] and Ferris and Hynes.[14] No effect on the blood pressure was obtained by Anderson [10] and by Apfelbach and Jensen.[15]

2. Polyuria was noted by Bradford,[2] Pässler and Heinecke,[3] Janeway,[6] Allen, Scharf and Lundin [11] and Apfelbach and Jensen.[15] Absence of polyuria was recorded by Bainbridge and Beddard,[4] Pearce,[5] Pilcher [8] and Mark.[12]

3. Practically all workers have reported a nitrogen retention in the blood.

4. Edema was never noted in partially nephrectomized animals.

5. Albuminuria and cylindruria were not studied in any detail by any of the foregoing investigators.

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14. Ferris, H. W., and Hynes, J. F.: Indirect Blood Pressure Readings in Dogs: Description of Method and Report of Results, J. Lab. & Clin. Med. **16**:597, 1931.

15. Apfelbach, C. W., and Jensen, C. R.: Experimental Renal Insufficiency in Dogs with Special Reference to Arterial Hypertension, J. Clin. Investigation **10**: 162, 1931.

EXPERIMENTAL METHODS

A Wistar strain of the albino rat was used in this work. These animals were raised and bred in our laboratory and were free from infection and parasitic infestation. The rat is extremely well adapted to the type of work to be reported, since it is notoriously hardy and resistant to operative infection. Furthermore, large numbers of biologically standardized animals can be studied.

6. Cardiac hypertrophy was noted by Pässler and Heinecke [3] and Mark and Geisendörfer.[12a] Bradford [2] and Pilcher [5] reported negative results.

The animals were anesthetized with ether; the hair over the left kidney region was clipped short; 95 per cent alcohol was poured over the area, and an incision was made directly into the peritoneal cavity. The left kidney was brought to the surface; loops of white cotton thread were placed tightly in position around both poles and then ligated off by closing the loops. By this method, from one half to three fourths of one kidney was removed from the circulation. In some cases the ligated poles were completely removed, but usually they were allowed to remain in situ. Very little hemorrhage accompanied this procedure. The ligated kidney was returned to its fossa, and the peritoneum, the muscle wall and the skin were approximated in two layers with a continuous suture of black silk. The whole procedure, including anesthesia, required from three to four minutes. One week later the right kidney was excised by a similar approach. The control animals were carried through the same operative procedure, the kidneys being exposed, handled and replaced. Other than dipping the instruments in 95 per cent alcohol before use and pouring alcohol over the skin, no aseptic precautions were taken. Following operation, there developed a few superficial abscesses, which cleared up after drainage. Neither peritonitis nor renal suppuration occurred in any animal. There was no noticeable reaction from the nonabsorbable thread used.

As a general rule, from six to eight animals of the same litter were placed in an adequately roomy cage. One control animal was allowed for every three or four nephrectomized rats. Immediately after the second operation all animals were placed on a diet containing 20 per cent dried extracted beef, 50 per cent dextrin, 16 per cent lard, 5 per cent standardized cod liver oil, 4 per cent salt mixture (Osborne and Mendel) and 5 per cent yeast. Food and water were always available.

The blood was obtained for analysis by clipping the tail of the anesthetized rat. The hemoglobin determinations were made by the Newcomer method. The Folin and Svedberg [16] micromethod was used for the determination of nonprotein nitrogen. The specific gravity of the serum, whole blood and urine was measured by the falling drop method of Barbour and Hamilton.[17]

Twenty-four hour specimens of urine were obtained for study by placing the rats in individual metabolism cages similar to those described by Levine and Smith.[18] Food was withdrawn twenty-four hours before the rat was placed in the

16. Folin, O., and Svedberg, A.: Micro Methods for the Determination of Non-Protein Nitrogen, Urea, Uric Acid, and Sugar in Unlaked Blood, J. Biol. Chem. **88**:85, 1931.

17. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, J. Biol. Chem. **69**:625, 1926.

18. Levine, H., and Smith, A. H.: A Cage Device for the Study of Ketosis and Nitrogen Metabolism on Small Animals, J. Lab. & Clin. Med. **11**:3, 1925.

metabolism cage in order to eliminate the possible effect of food metabolites on urinary excretion. The animal was then placed in the cage without water for twenty-four hours, and the urine for this period was collected. The volume was measured, the urine centrifugated and the specific gravity and albumin[19] determined.

Unfortunately no satisfactory method for following the blood pressure in the rat at frequent intervals could be devised. An attempt was made to apply the universally accepted clinical method of determining blood pressure by applying a small cuff connected with a clinical mercury manometer to the tail of the rat. The animal was anesthetized with ether, the cuff applied to the tail and the pressure increased above the expected blood pressure. The tail was cut with sharp scissors and immersed in a small beaker containing isotonic citrate solution. The pressure was then lowered until there was a marked spurt of blood from the tail artery. This point was extremely sharp in most animals. However, the blood pressure obtained by this method was extremely variable and much lower than that obtained by cannulating the carotid and therefore was abandoned. In moribund animals showing a carotid blood pressure of about 100 mm of mercury, it was frequently noted that the tail blood pressure would be about 20 mm. In animals showing a high blood pressure by the carotid method, it was usually found that the tail pressure was also high.

An effort was made to cannulate the tail artery, but this method was also unsatisfactory.

All blood pressure readings to be given were obtained by cannulating the carotid just before the animal was killed. The carotid was cannulated with needles of varying bores depending on the size of the vessel. A mercury manometer of about 0.7 mm. bore that was checked against a clinical manometer was used. The apparatus was similar to that used by Rous and Drury.[20] The system was filled with a solution of 5 per cent sodium citrate to prevent clotting The level of the mercury was adjusted to approximate the expected pressure in order to prevent loss of blood into the manometer. On separating the carotid of an hypertensive rat from the surrounding fascia, the vessel was found to be tortuous and would bulge from its normal position. The carotid arteries in these animals were markedly dilated, but no gross thickening or sclerosis was noted. Through observations of the carotid we were able by experience to estimate the blood pressure level with considerable accuracy. The animal was anesthetized with ether, the artery was isolated and cannulated, and pressure readings were begun within two minutes of the beginning of the anesthesia. Final readings were taken while there was a corneal reflex but before the animal awakened sufficiently to move.

The heart and kidney were removed immediately after death, weighed and either preserved for microscopic study[21] or used for the determination of the total solids. Care was taken to remove all of the blood from the heart cavities. A major part of the atria was removed in order to get rid of the first portion of the aorta. The kidneys were weighed after the remaining portions of the necrotic stumps had been removed. Total solid determinations of the heart and kidneys were made by drying for twenty-four hours at from 100 to 110 C. in a constant temperature oven.

19. Folin, Otto: A Laboratory Manual of Biological Chemistry, New York, D. Appleton & Company, 1926, p. 210.

20. Rous, P., and Drury, D. R.: Outlying Acidosis Due to Functional Ischemia, J. Exper. Med. **49**:435, 1929.

21. The histopathologic studies of the hearts and kidneys will be reported by Dr. J. Edwin Wood, Jr.

EXPERIMENTAL RESULTS

Stock Animals.—In order to test the reliability of our standards of measurements of the heart, kidneys and blood pressure in intact animals of different ages, weights and sex, a number of stock animals [22] were killed. The surface area was determined by the formula proposed by Lee [23] in which $S = 12.54 \times W^{0.60}$. The ratio of $\frac{\text{heart or kidney weight}}{\text{surface area}} \times 100$ has proved to be satisfactory in determining the relative changes in the weight of the heart or kidney, particularly of the heart. In table 1 it will be seen that the variations for the heart ratio were small, the average ratio being 0.177. The ratio for the kidneys was slightly more variable, with an average of 0.486. The limits of blood pressure in these animals ranged between 110 and 136 mm. of mercury.

TABLE 1.—*Stock Animals*

Rat	Weight, Gm.	Heart Weight, Gm.	Heart Weight / Surface Area × 100	Kidney Weight, Gm.	Kidney Weight / Surface Area × 100	Blood Pressure, Mm. Hg	Age at Death, Days
1	342	0.695	0.167	1.968	0.472	...	142
2	296	0.623	0.163	1.782	0.467	120	142
3	296	0.687	0.180	1.875	0.490	110	142
4	336	0.758	0.185	2.106	0.513	126	147
5	326	0.712	0.177	2.025	0.503	136	147
6	268	0.625	0.176	1.788	0.503	122	166
7	269	0.632	0.176	1.767	0.490	135	166
8	286	0.688	0.185	1.960	0.527	132	202
9	290	0.664	0.177	1.811	0.482	130	332
10	173	0.495	0.179	1.233	0.448	126	332
11	226	0.608	0.188	1.472	0.456	126	337
Average.....			0.177		0.486	126	

Control Animals.—As previously stated, the control animals were kept from three to two hundred and twenty-four days after laparotomy under identical conditions as the partially nephrectomized rats (table 2). The blood nonprotein nitrogen fluctuated between 30 and 54 mg. per hundred cubic centimeters (average, 38 mg.). The specific gravity of the serum varied between 1.0250 and 1.0290 (average, 1.0270). The carotid blood pressure varied between 106 and 145, with an average of 120 mm., which closely approximated the figures obtained by Durant [24] in the adult white rat. Since there was but one animal the blood pressure of which exceeded 140 mm., this figure was chosen as the upper limit of normal blood pressure.

22. The animals were fed a dog food, Bal Ra, prepared by the Valentine Meat Juice Company, Richmond, Va.

23. Lee, M. O.: Determination of the Surface Area of the White Rat with Its Application of the Expression of Metabolic Results, Am. J. Physiol. **89**:24, 1929.

24. Durant, R. R.: Blood Pressure in the Rat, Am. J. Physiol. **81**:679, 1927.

The specific gravity of the twenty-four hour specimens of urine collected for the concentration test showed variations from 1.0352 to 1.0733 (average, 1.0600). The specific gravity of the urine of the animals having access to food and water averaged about 1.0500. The

TABLE 2.—*Control Animals*

Rat	Weight, Gm.	Duration of Experiment, Days	Age at Death, Days	Hemoglobin, Gm. per 100 Cc.	Heart Weight: Surface Area × 100	Kidney Weight: Surface Area × 100	Blood Pressure, Mm.	Non-protein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume, Cc.	Specific Gravity	Albumin, Gm.
388	326	3	160	....	0.185	0.557	118	45	...	.....	.....
335	259	4	335	....	0.176	0.466	110	54	...	.....	.....
324	230	11	221	....	0.173	0.583	...	43	...	.....	.....
311	212	12	241	....	0.192	0.423	120	40	3.5	1.0391	.....
315	267	12	153	....	0.168	0.432	118	40	3.7	1.0428	.....
426	366	72	295	15.3	0.193	0.417	124	48	1.9	1.0710	.....
319	400	72	269	....	0.180	0.438	145	..	2.9	1.0671	.....
90	188	79	389	....	0.183	0.427	120	36	1.3	1.0631	.....
573	324	87	391	15.8	0.178	0.508	116	..	3.5	1.0543	.....
570	310	87	394	16.1	0.201	0.522	116	..	2.4	1.0724	.....
545	279	97	337	16.8	0.208	0.500	110	..	3.7	1.0367	0.006
543	252	97	337	12.7	0.185	0.537	116	33	2.4	1.0632	.....
547	226	97	256	15.0	0.202	0.536	106	34	3.6	1.0456	0.003
550	267	97	245	16.6	0.207	0.520	...	26	3.1	1.0484	.....
175	301	99	153	....	0.187	0.454	140	43	2.8	1.0449	.....
575	304	100	270	12.4	0.172	0.506	108	29	2.5	1.0540	.....
554	207	101	283	17.3	0.168	0.398	112	50	2.3	1.0512	.....
477	277	109	268	10.9	0.192	0.480	110	45	3.3	1.0409	.....
87	192	112	398	....	0.187	0.380	106	..	1.5	1.0631	.....
506	214	119	191	18.1	0.165	0.408	110	42	2.0	1.0582	.....
442	345	122	271	15.8	0.181	0.387	130	32	2.7	1.0586	.....
415	380	124	284	15.0	0.192	0.294	120	31	3.9	1.0470	0.003
20	192	133	334	....	0.166	0.400	112	48	1.4	1.0535	.....
19	225	133	326	....	0.172	0.437	126	47	1.1	1.0673	.....
23	160	136	327	....	0.178	0.442	120	40	1.5	1.0733	0.005
75	255	140	256	....	0.202	0.432	116	35	1.3	1.0705	.....
14	286	167	234	....	0.183	0.443	138	..	2.0	1.0605	.....
13	296	168	235	....	0.190	0.498	126	..	3.0	1.0617	.....
118	284	173	222	14.8	0.187	0.432	118	..	1.5	1.0692	.....
109	181	174	223	15.0	0.188	0.386	120	30	1.4	1.0642	.....
110	226	174	223	12.1	0.215	0.364	...	30	2.1	1.0607	.....
111	193	174	223	13.0	0.184	0.375	114	35	0.9	1.0621	.....
150	418	182	395	16.0	0.166	0.368	126	23	2.7	1.0518	.....
94	255	183	344	16.0	0.193	0.417	134	30	0.6	1.0708	.....
95	204	183	344	17.0	0.185	0.414	142	33	1.6	1.0665	.....
8	245	185	299	15.3	0.193	0.390	110	29	2.7	1.0624	0.003
61	195	187	268	14.5	0.177	0.411	140	37	1.2	1.0793	.....
52	241	198	485	....	0.184	.....	108	..	2.2	1.0617	.....
55	264	198	485	....	0.202	0.380	116	..	1.9	1.0606	0.004
96	248	200	415	15.2	0.184	0.417	120	..	2.8	1.0399	.....
135	302	207	246	14.8	0.205	0.455	118	33	2.5	1.0602	0.004
70	343	211	316	17.5	0.196	0.462	110	47	5.4	1.0352	0.001
61	340	212	283	18.2	0.209	0.477	...	43	2.0	1.0636	.....
41	237	212	398	16.5	0.190	0.408	120	43	2.3	1.0553	.....
62	281	212	283	19.0	0.170	0.364	120	48	1.7	1.0521	.....
35	366	213	520	15.3	0.200	0.423	136	50	4.0	1.0470	.....
5	232	213	495	15.8	0.200	0.437	...	..	1.7	1.0613	.....
6	385	219	307	16.8	0.186	0.421	112	48	1.8	1.0665	.....
58	332	219	307	17.3	0.204	0.465	...	34	2.7	1.0622	.....
169	251	224	294	....	0.180	.....	...	..	1.7	1.0650	.....
108	369	224	294	14.0	0.197	0.436	110	31	2.9	1.0547	.....
Average.....				15.5	0.191	0.438	120	38	2.4	1.0579	

albino rat can and normally does excrete a more concentrated urine than man. The twenty-four hour specimen of urine obtained during a concentration test varied in volume between 0.9 and 5.4 cc. (average, 2 cc.). As was to be expected, the specific gravity varied with the volume. The normal slight trace of albumin was most always found in the urine. The sediment was constantly negative for casts.

The $\frac{\text{heart weight}}{\text{surface area}} \times 100$ ratio ranged between 0.164 and 0.204 (average, 0.191). The kidney ratios were more variable. Total solid determinations of both the heart and the kidneys were almost constant, the former approximating 23 per cent and the latter 25 per cent.

An appreciable number of the control animals exhibited multiple lung abscesses, atelectasis or emphysema. In these there was a constant increase in the heart ratio, some reaching 0.250, but the blood pressure tended to be slightly lower (from 100 to 124; average, 113 in eleven rats) than in the healthy control animals. Therefore, animals with pulmonary disease were excluded.

Urinary and blood studies at frequent intervals showed that the results for an individual rat were fairly uniform over a considerable period of time. A typical case is shown in table 3.

TABLE 3.—*Protocol of Rat 41, Used as Control*

Time After Operation, Days	Weight, Gm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Hemoglobin, Gm. per 100 Cc.	Urine (24 Hour Specimen)		
				Volume, Cc.	Specific Gravity	Albumin, Gm.
0	240	..	....	...	.....	.....
18	...	40	....	...	.....	.....
29	246	..	....	...	.....	.....
62	250	38	....	...	.....	.....
88	...	..	....	2.1	1.0626	0.002
112	277	37	....	...	.....	.....
118	...	..	....	2.5	1.0426	.....
173	...	..	....	2.4	1.0462	0.001
176	258	33	17.3	...	.....	.....
211	...	..	....	2.3	1.0553	.....
212	237	43	16.5	...	.....	.....

Notes: Rat 41: male, aged 183 days. Killed April 16, 1931. Not sick; no pathologic changes. Blood pressure 120; heart ratio 0.190; total solids 22.9 per cent; kidney ratio 0.408; total solids 25.0 per cent; serum specific gravity 1.0270; specific gravity of whole blood 1.0627; microscopically, urine negative.

The Early Postoperative Stage in Apparently Well, Partially Nephrectomized Animals.—Definite nitrogen retention and pronounced polyuria were noted during the first few days after the second operation. Within two weeks, however, there was usually a decreased volume of urine. About the third week, the degree of renal damage apparently determined whether the nitrogen retention and polyuria were to be decreased or increased. The loss of weight noted in these animals was probably due for the most part to the frequent concentration tests made. Table 4 contains the details of changes in representative animals during the early postoperative stage.

These rats showed no signs of listlessness, emaciation or ruffled hair and, as far as could be determined by observation, were in relatively good condition; apparently they might have lived for from one to six months. A number of rats showing a marked renal insufficiency, as

judged by the concentration test, were killed at various periods shortly after the second operation in order to study the blood pressure changes and the heart and kidney weights during this stage (table 5). A marked renal insufficiency accompanied by varying degrees of nitrogen retention was characteristic. Renal hypertrophy was noticeable after the second week. The degree of albuminuria did not differ appreciably

TABLE 4.—*Protocol of Rats 606, 585 and 603*

Time After Operation, Days	Weight, Gm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Hemoglobin, Gm. per 100 Cc.	Urine (24 Hour Specimen)		
				Volume, Cc.	Specific Gravity	Albumin, Gm.
Rat 606:						
0	343	...	....	....	.....	.....
2	...	...	....	12.7	1.0181	0.014
5	...	127	17.0	....	.....	.....
9	284	43	14.9	....	.....	.....
Rat 585:						
0	340	...	....	....	.....	.....
1	...	...	....	8.3	1.0201	0.011
8	...	...	....	8.7	1.0200	.....
10	286	62	....	....	.....	.....
29	...	...	....	17.3	1.0187	0.086
31	...	99	12.8	....	.....	.....
35	269	82	10.0	....	.....	.....
Rat 603:						
0	305	...	....	....	.....	.....
2	...	...	....	17.4	1.0183	0.014
5	...	127	17.5	....	.....	.....
13	...	...	....	10.2	1.0189	.....
15	273	50	....	....	.....	.....
34	...	...	....	9.6	1.0259	0.009
37	...	44	11.0	....	.....	.....
41	318	73	10.2	....	.....	.....

Notes: Rat 606: Two thirds of left kidney removed on May 8, 1931; right kidney removed on May 13. Killed on May 22. Not sick; no pathologic changes. Blood pressure 122; heart ratio 0.180; kidney ratio 0.109.

Rat 585: Two thirds of left kidney removed on May 8, 1931; right kidney removed on May 18. Killed on June 24. Not sick; no pathologic changes. Blood pressure 150; heart ratio 0.215; kidney ratio 0.213.

Rat 603: Two thirds of left kidney removed on May 8, 1931; right kidney removed on May 13. Killed on June 24. Not sick; no pathologic changes. Blood pressure 118; heart ratio 0.216; kidney ratio 0.162.

TABLE 5.—*The Early Postoperative Stage in (Apparently) Well, Partially Nephrectomized Animals*

Rat	Weight, Gm.	Duration of Experiment, Days	Age at Death, Days	Hemoglobin, Gm. per 100 Cc.	Heart Weight	Kidney Weight	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test		
					Surface Area × 100	Surface Area × 100			Volume, Cc.	Specific Gravity	Albumin, Gm.
584	288	1	238	....	0.202	.....	...	..	11.0	1.0168	0.017
569	171	6	275	14.1	0.177	.....	...	119	14.0	1.0167	0.006
568	146	6	275	14.3	0.162	.....	...	..	11.0	.....	.....
607	228	9	332	12.0	0.209	0.133	102	75	23.2	1.0095	0.005
606	284	9	332	14.9	0.180	0.109	122	43	12.7	1.0181	0.014
605	269	9	332	10.5	0.162	0.068	112	88	16.3	1.0132	0.008
432	98	10	154	....	0.202	0.084	147	218	....	.....	.....
307	205	12	215	....	0.174	0.215	126	..	16.5	1.0152	.....
585	299	37	274	10.0	0.215	0.313	150	82	17.3	1.0187	0.086
587	329	37	274	16.8	0.208	0.218	134	78	11.2	1.0246	0.012
Average					0.189						

from the normal. Hypertension as early as the tenth day without cardiac hypertrophy was found in only one case. Similar data as regards nitrogen retention, polyuria, low specific gravity, albuminuria and renal hypertrophy were obtained in a number of animals excluded on account of pulmonary disease.

TABLE 6.—*The Late Postoperative Stage in Apparently Well, Partially Nephrectomized Animals*

Rat	Weight, Gm.	Duration of Experiment, Days	Age at Death, Days	Hemoglobin, Gm. per 100 Cc.	Heart Weight / Kidney Weight: Surface Area × 100	Heart Weight / Kidney Weight: Surface Area × 100	Blood Pressure, Mm.	Non-protein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume, Cc.	Twenty-Four Hour Urine Concentration Test: Specific Gravity	Twenty-Four Hour Urine Concentration Test: Albumin, Gm.
425	292	43	267	12.2	0.247	0.211	172	106	14.8	1.0121	0.024
428	206	72	295	14.5	0.203	0.178	152	58	4.6	1.0302	0.006
429	344	72	319	13.3	0.218	0.243	120	67	9.4	1.0226	0.077
430	180	72	319	11.1	0.205	0.175	152	39	3.7	1.0274	.....
534	252	94	220	14.6	0.244	0.335	...	127	19.2	1.0173	0.128
578	200	98	269	16.1	0.217	0.325	152	83	14.5	1.0182	0.171
455	362	105	333	16.8	0.205	0.360	187	..	14.3	1.0222	0.272
504	245	112	191	14.9	0.213	0.370	180	91	13.0	1.0247	0.218
501	303	112	185	11.3	0.221	0.445	156	100	20.2	1.0236	0.131
508	173	112	191	12.6	0.227	0.349	162	87	10.3	1.0202	0.197
465	328	113	320	12.7	0.258	0.502	180	133	20.3	1.0175	0.089
505	188	115	192	11.7	0.265	0.412	200	197	12.7	1.0203	0.149
507	160	115	192	11.6	0.228	0.461	196	114	11.3	1.0198	0.173
466	356	116	355	12.0	0.238	0.560	196	111	19.0	1.0190	0.148
433	303	122	257	16.7	0.244	0.533	192	121	16.5	1.0184	0.204
418	167	124	284	13.7	0.222	0.210	166	60	7.4	1.0219	0.096
414	308	124	284	12.8	0.218	0.212	162	38	7.7	1.0312	0.005
166	248	170	238	13.0	0.248	0.498	214	133	13.0	1.0203	0.151
112	202	173	223	12.1	0.179	0.277	126	..	5.3	1.0387	.....
115	207	173	223	11.1	0.205	0.176	170	133	11.2	1.0183	0.058
114	216	173	223	14.1	0.176	0.222	...	..	8.0	1.0189	0.039
116	209	173	222	15.3	0.164	0.228	116	50	2.0	1.0513	.....
113	224	173	223	12.5	0.164	0.187	118	..	3.7	1.0357	.....
108	234	174	223	13.2	0.204	0.226	150	69	7.7	1.0275	0.132
102	229	174	223	13.9	0.210	0.308	172	103	8.7	1.0280	0.187
106	208	174	223	14.6	0.179	0.208	124	58	3.8	1.0394	.....
98	206	180	289	15.5	0.211	0.330	152	96	9.8	1.0219	0.063
147	304	182	395	12.8	0.218	0.316	168	93	11.0	1.0204	0.122
91	225	183	344	17.0	0.188	0.222	...	51	3.2	1.0390	.....
55	178	187	268	16.0	0.190	.....	128	68	2.0	1.0506	.....
143	272	197	470	13.7	0.198	0.223	140	79	8.8	1.0263	0.151
66	393	211	316	13.2	0.231	0.603	156	112	16.8	1.0172	0.033
57	168	212	283	11.1	0.256	0.380	190	180	14.1	1.0163	0.213
58	172	212	283	12.0	0.249	0.418	186	121	9.2	1.0171	0.089
3	243	213	495	11.3	0.263	0.417	170	62	10.5	1.0189	0.067
37	296	213	525	15.0	0.267	0.336	220	132	14.1	1.0151	0.056
5	240	216	259	15.6	0.212	.....	160	112	11.3	1.0231	0.136
162	352	216	294	12.8	0.258	0.610	160	74	10.8	1.0268	0.275
7	398	219	307	14.9	0.200	0.193	130	48	9.6	1.0262	0.204
164	261	224	294	13.4	0.190	0.273	110	61	7.9	1.0251	0.114
163	297	225	293	11.8	0.310	0.370	230	174	13.1	1.0206	0.295
Average					0.221		164				

The Late Postoperative Stage in Apparently Well, Partially Nephrectomized Animals.—This group includes partially nephrectomized rats that were killed at various intervals beginning forty-three days after the second operation (table 6). In order to eliminate the effects of pulmonary disease and anemia [25] on heart size, only those animals with normal lungs and a hemoglobin of more than 11 Gm. per

25. Forman, M. B., and Daniels, A. L.: Effect of Nutritional Anemia on the Size of the Heart, Proc. Soc. Exper. Biol. & Med. **28**:479, 1931.

hundred cubic centimeters of blood are listed in this group. The data presented were obtained shortly before or at the time of death. Observations were made at frequent intervals on each rat so that fairly complete individual histories were available.

The nonprotein nitrogen ranged from 50 to 197 mg. per hundred cubic centimeters, agreeing roughly with the degree of renal insufficiency as estimated by the urinary volume and specific gravity. It was of special interest to note the degree of nitrogen retention that could be reached and maintained in an individual rat without appreciable evidences of toxemia.

Despite the fact that the amount of kidney tissue removed was approximately the same in all cases, there was a marked individual difference in the renal function. Furthermore, there seemed to be no relationship between the degree of kidney hypertrophy and kidney function. Histologic study may explain this apparent discrepancy. The specific gravity of the urine varied between 1.0121 and 1.0556. It was not unusual to obtain from 20 to 25 cc. of urine during a concentration test. These figures are more striking when it is remembered that the maximum output in the control animals was 5.4 cc. In all these animals, the albuminuria was initially within normal limits, but after periods varying from one to two months a progressive increase was noted. An albuminuria represented by 0.295 Gm. of dried protein was the maximum figure obtained. Although casts eventually were found in the urine of all animals having a deficient kidney function, the time of their initial appearance could not be predicted.

The specific gravity of the blood serum was within the normal range. The specific gravity of the whole blood paralleled the hemoglobin values.

The carotid blood pressure readings were striking. They varied from 110 to 230 mm., with most of them well above the upper limits of normal. We found that animals with marked sustained renal insufficiency invariably showed high blood pressure, but that the degree of nitrogen retention could not be used as a criterion for the prediction of hypertension.

In practically all cases of hypertension, the $\frac{\text{heart weight}}{\text{surface area}}$ ratio was increased. In addition definite thickening of the left ventricular wall was noted in many of them. No other gross cardiac abnormality was seen in this group. The percentage of total solids in the hypertensive hearts was no different from that of the controls, thus eliminating the possible effect of water on the increased weight.

The variations in hypertrophy of the kidney were shown by the $\frac{\text{kidney weight}}{\text{surface area}}$ ratios. Immediately after the second operation, the kidney ratio was as low as 0.075; hypertrophy in the kidney remnant progressed rapidly, and in many cases its ratio exceeded the control value of 0.438

for both kidneys. The total solids in these hypertrophied kidneys varied from 14.2 to 23 per cent, the majority being between 15 and 21 per cent. The hypertrophied kidneys had a fairly typical appearance. The capsule was noticeably thickened and at times adherent. The surface was pale, edematous and yellowish brown. Varying amounts of the ligated stumps remained, depending on the duration of the experiment. Soon after the operation the stumps consisted of an encapsulated mass

TABLE 7.—*Protocol of Rat 57*

Time After Operation, Days	Weight, Gm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Hemoglobin, Gm. per 100 Cc.	Urine (24 Hour Specimen)		
				Volume, Cc.	Specific Gravity	Albumin, Gm.
0	103	..	....	...	.....	.....
53	151	68	....	...	.....	.....
62	156	93	....	...	.....	.....
87	....	..	....	5.5	1.0200	0.000
105	...	..	....	7.5	1.0296	.....
111	170	78	....	...	.....	.....
136	....	..	....	6.4	1.0276	.....
175	179	..	13.9	...	.....	.....
173	....	..	....	12.8	1.0158	0.096
204	....	..	....	14.1	1.0163	0.213
212	168	180	11.1	...	.....	.....

Notes: Rat 57: Female, aged 70 days. Two thirds of left kidney removed on Sept. 11, 1930; right removed on September 18. Killed on April 17, 1931. Not sick; no pathologic changes. Blood pressure 190, carotid markedly dilated and tortuous; heart ratio 0.256, total solids 22.9 per cent; kidney ratio 0.380, total solids 17.2 per cent; serum specific gravity 1.0260; specific gravity of whole blood 1.0549; urinary sediment contained an occasional granular cast.

TABLE 8.—*Protocol of Rat 163*

Time After Operation, Days	Weight, Gm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Hemoglobin, Gm. per 100 Cc.	Urine (24 Hour Specimen)		
				Volume, Cc.	Specific Gravity	Albumin, Gm.
0	92	..	....	...	.....	.....
37	....	..	....	3.4	1.0416	0.005
86	234	80	....	...	.....	.....
109	....	..	....	5.8	1.0296	.....
141	....	..	....	14.8	1.0235	0.175
172	224	114	....	...	.....	.....
207	....	..	....	13.1	1.0206	0.295
225	207	174	11.8	...	.....	.....

Notes: Rat 163: Female, aged 66 days. Two thirds of left kidney removed on Oct. 28, 1930; right removed on November 3. Killed on June 15, 1931. Not sick; no pathologic changes. Blood pressure 230, carotid markedly dilated and tortuous; heart ratio 0.310; kidney ratio 0.370; urinary sediment contained many hyaline casts.

of necrotic cheeselike material. This was gradually absorbed until a small hard mass remained, which in turn finally disappeared entirely. On section the kidney was pale, edematous and yellowish brown with an indistinct cortex.

An occasional animal had a small amount of fluid in the peritoneal or the pleural cavities or in both. "Wet" subcutaneous tissue was encountered in a few rats. Since these findings were so unusual in our experimental animals, they were not considered a part of the typical syndrome encountered in experimental kidney insufficiency

Tables 7, 8 and 9 illustrate the most typical changes in individual animals in this group. The urinary changes recorded in table 7 are most representative of the majority of partially nephrectomized animals. There were a gradual decrease in the specific gravity and an increase in the urinary volume. The albuminuria became pronounced after the second month. The nonprotein nitrogen was elevated but not exceedingly high. Table 8 shows an animal which soon after operation had an apparently normal kidney function, but after a few months the

TABLE 9.—*Protocol of Rat 425*

Time After Operation, Days	Weight, Gm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Hemoglobin, Gm. per 100 Cc.	Urine (24 Hour Specimen)		
				Volume, Cc.	Specific Gravity	Albumin, Gm.
0	285	...	....	....	.....	.....
9	...	...	....	13.6	1.0166	0.012
36	...	...	....	14.8	1.0121	0.034
40	292	106	12.2	....	.....	.....

Notes: Rat 425: Male, aged 214 days. Two thirds of left kidney removed on Feb. 2, 1931; right removed February 11. Killed March 23. Animal slightly weak; no pathologic changes. Heart ratio 0.247; kidney ratio 0.211; blood pressure 172, carotid markedly dilated and slightly tortuous; kidney stumps markedly atrophied.

TABLE 10.—*Partially Nephrectomized Animals Killed While in Good Health Showing Moderate Anemia*

Rat	Weight, Gm.	Duration of Experiment, Days	Age at Death, Days	Hemoglobin, Gm. per 100 Cc.	Heart Weight Surface Area × 100	Kidney Weight Surface Area × 100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test		
									Volume, Cc.	Specific Gravity	Albumin, Gm.
593	140	40	120	8.0	0.197	0.143	142	179	12.6	1.0146	0.013
596	168	42	310	9.9	0.190	0.287	144	89	11.6	1.0187	0.009
601	233	42	207	7.6	0.222	0.187	126	94	11.7	1.0193	0.053
368	213	69	193	9.3	0.290	0.270	166	189	16.2	1.0129	0.016
559	111	72	250	7.0	0.245	0.236	194	266	11.4	1.0149	0.022
552	278	96	244	9.7	0.258	0.422	170	133	17.5	1.0166	0.112
531	250	100	250	8.3	0.216	0.330	134	87	15.8	1.0195	0.147
553	185	101	283	8.9	0.187	0.213	124	104	10.5	1.0173	0.056
480	278	109	268	8.9	0.219	0.362	156	98	15.3	1.0209	0.121
479	262	109	269	9.7	0.261	0.383	178	154	22.0	1.0205	0.455
364	214	115	239	6.6	0.260	0.281	214	218	14.6	1.0165	0.073
532	256	123	231	6.5	0.254	0.430	186	220	20.8	1.0125	0.043
439	338	133	272	9.5	0.296	0.255	184	77	15.3	1.0196	0.250
Average					0.238		163				

characteristic "nephritic" changes in specific gravity, urinary volume and albuminuria developed. The animal in table 9 was chosen to illustrate a continued high urinary volume and a low specific gravity from the time of the second operation. In this case there was hypertension without marked albuminuria.

The Late Postoperative Stage in Partially Nephrectomized Animals with Anemia.—Partially nephrectomized animals killed while in good health but showing a moderate anemia are grouped in table 10. The outstanding difference between this and the preceding group is the large

volume of urine with uniformly low specific gravity. Despite the anemia there seemed to be a fairly good correlation between the blood pressure and the heart ratio. It should be pointed out that the degree of anemia was not so great as noted in cases of nutritional anemia.[25] An occasional rat showed poor renal function without hypertension or cardiac hypertrophy. The tremendous albuminuria noted in some of the partially nephrectomized animals is worthy of mention. Rat 479 excreted 0.455 Gm. of protein in twenty-four hours, which represents about one-half its total circulating plasma protein. The absence

TABLE 11.—*Partially Nephrectomized Animals Living Less Than Forty Days and Killed While Acutely Ill*

Rat	Weight, Gm.	Duration of Experiment, Days	Age at Death, Days	Heart Weight / Surface Area × 100	Kidney Weight / Surface Area × 100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	24 Hour Urine Concentration Test: Volume, Cc.	24 Hour Urine Concentration Test: Specific Gravity	Degree of Illness
390	173	3	170	0.168	0.058	104	250	....	.....	4+
389	227	3	165	0.200	0.097	108	308	....	.....	4+
387	240	3	169	0.194	0.064	120	320	....	.....	4+
380	238	3	241	0.172	0.098	96	111	....	.....	4+
381	259	3	219	0.193	0.067	138	267	....	.....	4+
399	147	3	122	0.196	0.087	116	364	....	.....	4+
303	135	5	151	0.208	0.091	110	445	....	.....	4+
301	164	5	151	0.194	0.123	100	325	....	.....	4+
401	255	7	141	0.210	0.121	90	226	....	.....	3+
400	176	7	141	0.214	0.092	94	454	....	.....	4+
195	165	7	279	0.220	0.093	94	348	....	.....	4+
427	151	8	234	0.166	0.063	96	276	....	.....	3+
358	273	10	345	0.223	0.114	110	348	....	.....	3+
308	183	12	215	0.172	0.149	80	250	17.0	1.0135	4+
309	207	12	215	0.168	0.121	110	210	14.8	1.0150	3+
351	153	18	163	0.205	0.114	92	416	....	.....	4+
459	186	19	270	0.187	0.125	90	370	14.7	1.0128	3+
317	166	32	220	0.182	0.151	110	...	16.0	1.0144	2+
Average				0.193	0.101	103	310			

of any correlation between blood pressure and nitrogen retention is again emphasized in this group.

Partially Nephrectomized Animals Killed While Ill.—Animals included in this group were killed because of their poor condition and imminent death. The signs 1 to 4 + were used as a rough method of indicating the degree of illness of a rat. A 4 + animal was practically moribund at the time it was killed. The 3 + and 2 + animals probably could not have survived more than from twenty-four to forty-eight hours. The 1 + animals were in fairly good condition, but showed ruffled hair and an acute loss of weight.

Partially Nephrectomized Animals Acutely Ill.—All rats living less than forty days have been included in this group (table 11). The heart ratios were practically normal despite the fact that most of these animals lost between 40 and 50 per cent of their body weight. The results were

only approximate since the surface area formula used was not intended for animals showing acute loss in body weight. The kidney ratios were relatively low when compared to those of well animals killed during this same period. Obviously too much kidney tissue had been removed. The blood pressures were markedly lowered, but were comparatively high considering the moribund condition of the rats. This seemed to be characteristic of all the animals suffering from acute cachexia. The nonprotein nitrogen in this group represented the highest degree of

TABLE 12.—*Partially Nephrectomized Animals Becoming Ill After Forty Days*

Rat	Weight, Gm.	Duration of Experiment, Days	Age at Death, Days	Hemoglobin, Gm. per 100 Cc.	Heart Weight / Surface Area × 100	Kidney Weight / Surface Area × 100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	24 Hour Urine Concentration Test: Volume, Cc.	Specific Gravity	Albumin, Gm.	Degree of Illness
549	159	49	195	....	0.230	0.204	30	...	17.4	1.0160	0.035	4+
422	193	56	221	11.6	0.279	0.217	118	129	10.2	1.0150	0.010	2+
363	224	59	183	10.5	0.242	0.286	102	89	6.5	1.0244	0.119	4+
572	202	60	367	16.5	0.232	0.308	175	200	16.2	1.0136	0.028	4+
471	202	62	245	13.3	0.252	0.228	180	131	19.3	1.0148	0.027	2+
546	217	67	224	8.9	0.262	0.349	...	171	18.3	1.0162	0.128	3+
556	177	68	250	15.3	0.229	0.218	104	59	9.8	1.0243	.....	3+
569	282	69	379	17.0	0.226	0.340	90	123	10.4	1.0245	0.053	4+
323	238	70	277	14.8	0.286	0.210	126	129	14.4	1.0167	0.167	4+
370	208	72	195	6.6	0.270	0.143	154	...	17.8	1.0120	0.005	3+
330	159	72	220	11.2	0.246	0.282	166	125	9.6	1.0214	.....	3+
436	272	74	125	15.6	0.252	0.389	...	72	6.5	1.0312	0.008	4+
558	149	83	262	14.9	0.220	0.200	100	...	11.5	1.0150	0.128	4+
576	174	90	260	12.2	0.234	0.252	172	168	13.2	1.0194	0.156	3+
478	228	91	246	9.4	0.287	0.287	156	220	25.7	1.0143	0.082	2+
481	208	93	248	13.8	0.289	0.251	165	221	20.0	1.0147	0.084	3+
467	264	96	302	13.9	0.263	0.453	204	168	20.4	1.0168	0.153	4+
535	161	97	229	10.8	0.245	0.282	170	200	11.3	1.0167	0.135	2+
50	163	105	391	....	0.324	0.316	160	260	10.8	1.0135	.....	4+
57	150	118	199	....	0.252	0.262	152	135	9.9	1.0169	.....	4+
59	144	121	201	....	0.238	0.293	174	122	7.0	1.0226	.....	4+
42	217	140	336	....	0.298	0.359	140	88	8.5	1.0235	.....	4+
4	180	141	419	....	0.266	0.300	70	204	11.0	1.0143	.....	2+
137	179	162	200	12.0	0.231	0.156	...	...	15.8	1.0159	0.054	3+
56	187	197	270	11.5	0.226	0.500	...	76	6.6	1.0291	0.170	4+
97	158	200	415	12.9	0.308	0.410	190	96	12.7	1.0176	0.117	4+
40	236	211	395	9.0	0.302	0.450	178	...	12.4	1.0182	0.170	2+
39	251	212	398	17.0	0.276	0.292	55	222	18.5	1.0180	0.275	3+
Average.....					0.259	0.294		148		1.0180		

nitrogen retention encountered in our experiments. When concentration tests were done, marked polyuria and low specific gravity resulted.

Partially Nephrectomized Animals Becoming Ill After Forty Days.—Data for these animals are listed in table 12. After maintaining or increasing their weights for various lengths of time, they generally showed a marked and rapid loss of weight. During the terminal stage these rats became sluggish, with ruffling of their hair and rapid respiration. Muscular tremors were usually present, and occasional convulsions were seen. Partial paralysis of the hind legs was observed in a few animals. The same syndrome was noted in many of the "acutely ill" animals already discussed.

The nonprotein nitrogen was uniformly elevated but showed wide variations. The urinary volumes and specific gravities indicated a

consistently low renal function. The blood pressures were extremely variable. The heart ratio was uniformly well above normal, and the carotid arteries were invariably dilated; these two findings, in our experience with less affected rats, were characteristic of hypertension. Therefore, we think that all of the animals in this group would have shown hypertension at some time had repeated blood pressure observations been possible during the postoperative period. We believe that the low blood pressures were explicable on the basis of the imminency of death. It must be pointed out that many of these rats lost a great deal of weight during the terminal period, thus causing our surface area measurements to be merely approximate. However, a comparison with the heart ratios in table 11 leaves no doubt concerning the question of cardiac hypertrophy.

COMMENT AND CONCLUSIONS

The variations in the minimum quantities of kidney tissue needed by various species are of interest. It appears from work done by previous investigators that dogs die if more than three fourths of the kidney tissue is removed.[26] The goat apparently lives in good health with but one fifth of one kidney.[11] The rabbit seems incapable of surviving if more than from 60 to 70 per cent of the renal tissue is excised.[10] The removal of three fourths of the kidney substance in the cat is followed by death within a relatively short time.[4] Rats with one half of one kidney have a normal kidney function, but a sixth of the total kidney tissue, in our experience, is the minimum compatible with life.

Our results are in accord with those of Pässler and Heinecke,[3] who observed that ischemic kidney tissue disappeared and that it had no relation to elevation of the blood pressure. Although Cash [9] concluded that the preliminary rise of blood pressure in the dog shortly after partial nephrectomy was dependent on the presence of necrotic tissue, in our work the hypertension appearing after an appreciable interval was not affected by the removal or retention of the ligated tissue.

Impaired renal function, characterized by polyuria and nitrogen retention, was encountered in the majority of partially nephrectomized rats shortly after the second operation. The degree of polyuria in animals showing no indication of increased blood pressure was often as great as that encountered in hypertensive rats. Hence it was thought that the increased flow of urine of low specific gravity was not solely dependent on the presence of hypertension. The polyuria, so striking in a number of animals immediately after the second operation, was, in the absence of hypertension, probably due to an increased permeability

26. Bradford (footnote 2). Pässler and Heinecke (footnote 3). Allen, Scharf and Lundin (footnote 11).

of the glomerulus. When pathologic changes in the renal tissue (as judged by the increases in albuminuria, polyuria and nonprotein nitrogen in the apparently well animal) became obvious, an increase in the blood pressure was generally noted (table 6). The assumption seems warranted that in partially nephrectomized rats with renal insufficiency, an increase in blood pressure is necessary to maintain an increased volume of urine in order to excrete metabolites in lower concentration. Apparently this compensating mechanism fails when there is excessive damage to the remaining renal parenchyma (table 12), just as it fails when too much renal tissue is removed (table 4). Our evidence indicates that the polyuria in the partially nephrectomized rat, reaching the stage of chronic renal insufficiency, depends to a great extent on an increased blood pressure.

On the basis of perfusion experiments and theoretical consideration, Verney [27] postulated as follows: "In the whole animal, therefore, it would not be surprising to find that when this second reserve of the kidney was exhausted and all the renal units were receiving the maximum stimulus which under the circumstances could be given them, and yet were unable to cope with the work thrust upon them there should be a call for external aid in the form of a rise in the general blood pressure." This idea is certainly supported by our experimental data.

Nitrogen retention, although to a varying degree, characterized the results obtained in partially nephrectomized rats in this series. The highest nonprotein nitrogen invariably occurred in animals becoming ill shortly after the second operation; a value of 454 mg. was obtained in one case. The average results were higher than those seen in the completely nephrectomized rat.[28] This difference can probably be explained on the basis of a greater duration of time for the accumulation of metabolites in the partially nephrectomized rat. A marked increase in nitrogen retention was invariably indicative of the terminal stage. However, the severity of the illness was not in all cases comparable to the degree of nitrogen retention, since many moribund animals showed comparatively low concentrations of nonprotein nitrogen. Furthermore, a chronic moderate elevation of the nonprotein nitrogen had no demonstrable effect on the general health of the animal.

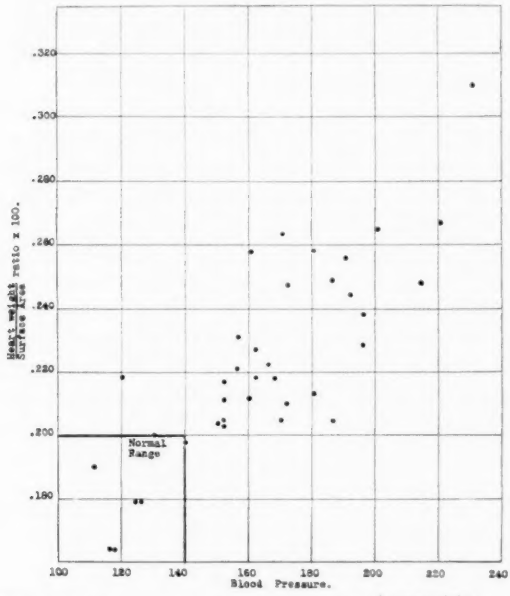
Pässler and Heinecke [8] stressed their failure to obtain hypertension in dogs becoming cachectic shortly after or during the course of their repeated operations. The necessity of good health was emphasized as a factor in the development of a high blood pressure. Our findings

27. Verney, E. B.: Polyuria in Chronic Nephritis, Lancet **1**:751, 1929.

28. Chanutin, A., and Silvette, H.: A Study of Creatine Metabolism in the Nephrectomized White Rat, J. Biol. Chem. **85**:179, 1929.

verify these conclusions. In our experiments, those animals losing weight and developing marked evidences of "uremia" showed definitely lower blood pressures than the controls (table 11).

It has been pointed out that but two groups of investigators [30] have been able to produce hypertension associated with cardiac hypertrophy as a result of partial nephrectomy. The data in table 6 have been used to show the relationship between hypertension and the $\frac{\text{heart weight}}{\text{surface area}}$ ratio (see the accompanying chart). A statistical analysis of these data by



Relationship between hypertension and the $\frac{\text{heart weight}}{\text{surface area}}$ ratio.

the use of the Pearson product-moment formula yielded a correlation coefficient (r) of 0.86 ± 0.028. This means that there is a high positive relationship between the $\frac{\text{heart weight}}{\text{surface area}}$ ratio and blood pressure. Such a finding must lead to the conclusion that in these experiments cardiac hypertrophy is a constant and proportional accompaniment of hypertension. As far as we know, this is the first experimental demonstration of such a relationship. We believe that the hypertension and cardiac hypertrophy obtained in these experiments are definitely dependent on the renal insufficiency produced by partial nephrectomy.

30. Pässler and Heinecke (footnote 3). Mark and Geisendörfer (footnote 12a).

SUMMARY

By reducing kidney tissue to a minimum compatible with life, chronic renal insufficiency has been reproduced with constancy in a sufficient number of rats. The striking features of this study were the progressive development of polyuria, albuminuria, nitrogen retention, renal hypertrophy, hypertension and cardiac hypertrophy. Edema did not appear, the specific gravity of the serum was constant, and there was a tendency toward a moderate anemia.

ACUTE BACTERIAL ENDOCARDITIS DUE TO BACTERIUM ACIDI-LACTICI

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While bacterial endocarditis is common, endocarditis due to Bacterium acidi-lactici appears to be unique; at least, I have found no other case in the literature. Winslow [1] considered this micro-organism to be a separate species in the Bact. coli group, although Topley and Wilson [2] regarded it as one of the varieties of Bact. coli. Ford [3] placed it in the Bact. mucosum-capsulatum group, and suggested for it the name Bact. duodenale.

It was first described by Hueppe [4] in 1884. It resembles the other bacilli of the colon group. It is gram-negative, nonmotile and nonsporulating. It forms indole, reduces nitrates, coagulates milk and does not liquefy gelatin. It is found in milk, water and the intestinal tract of man. It is distinguished from other types of Bact. coli, from Bact. lactis-aerogenes, and from Friedländer's bacillus by its characteristic sugar fermentations. It ferments dextrose, lactose, adonite and mannite, but does not ferment saccharose, dulcite, salicin, inosite, raffinose, xylose or inulin. It does not produce so much gas as Bacillus lactis-aerogenes.[5] At the Presbyterian Hospital it is routinely differentiated from the other organisms in the group by its lack of power to ferment saccharose and salicin.

The only example of infection with Bact. acidi-lactici found in the available literature was reported by Ray [5] in 1923: a case of meningitis in which the micro-organism was isolated from the blood and spinal fluid. It is possible that Bact. acidi-lactici infections in man may have been reported among the cases of Bact. coli sepsis. Ray attributed this

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1. Winslow, E.; Kligler, E., and Rothberg, A.: Studies on Colon-Typhoid Group of Bacteria, J. Bact. **4**:429, 1919.

2. Topley, W. W. C., and Wilson, G. S.: Principles of Bacteriology and Immunity, New York, William Wood & Company, 1929, vol. 1, p. 425.

3. Ford, W. W.: Text Book of Bacteriology, Philadelphia, W. B. Saunders Company, 1927, p. 556.

4. Hueppe, F.: Untersuchungen über die Versetzungen der Milch durch Microorganismen, Arb. a. d. k. Gsndhtsamte. **2**:309, 1884.

5. Ray, H. M.: Fatal Case of Bacillus Acidi Lactici Meningitis, J. Lab. & Clin. Med. **8**:260, 1923.

to the use of impure carbohydrate in making the fermentation tests and to the fact that the micro-organisms occasionally lose their original fermentative powers when cultured.

Several cases of endocarditis due to Bact. coli have been reported (Lenhartz,[6] Horder,[7] Norris,[8] Jochmann,[9] Summe,[10] Jacob[11]).

It is a strange coincidence that two cases of endocarditis due to Bact. acidi-lactici should have occurred in the Presbyterian Hospital within a short time of each other.

CASE 1.—The patient, R. B., was admitted on Nov. 8, 1930, to the genito-urinary service of Dr. Squier, Dr. George Cahill attending.[12] His complaints were frequency of micturition and nocturia. His family history was not important. The only previous illness of interest was a mastoiditis with operation two and one-half years before. The mastoid had to be reopened in two weeks, but it healed well with no evidence of residual infection. His present illness began abruptly, two days before admission, with marked frequency of urination, nocturia and fever. The temperature was 102 F., the pulse rate 96, respirations 20 and blood pressure 144 systolic and 60 diastolic. The patient was an elderly, obese man, acutely ill. The chest and abdomen were normal on examination. The heart sounds were of fair quality, and no murmurs were heard. The prostate was prominent, soft and tender. There was slight hematuria after the rectal examination.

The following laboratory observations were made:

Blood Count

	Red Blood Cells	White Blood Cells	Polymorpho-nuclears, per Cent
Nov. 12	3,700,000	10,000	86
Nov. 19	3,400,000	27,850	92
Dec. 1	3,450,000	15,000	91
Dec. 10	3,000,000	26,400	89

Urinalysis showed: cloudy, acid urine; specific gravity, 1.016; albumin, ++, diacetic acid, ++++; many red blood corpuscles and white blood corpuscles. The blood urea content was 44.1 mg. per hundred cubic centimeters; the carbon dioxide content, 71 per cent by volume.

6. Lenhartz, H.: Die septischen Erkrankungen, Vienna, A. Hölder, 1903.

7. Horder, T. J.: Infective Endocarditis with an Analysis of 150 Cases with Special Reference to the Chronic Form of the Disease, Quart. J. Med. **2**:289, 1909.

8. Norris, G. W. Studies in Cardiac Pathology, Philadelphia, W. B. Saunders Company, 1911, p. 26.

9. Jochmann, G.: Ueber Endocarditis septica, Berl. klin. Wchnschr. **49**:436 1912.

10. Summe, quoted by Kraus, F., and Brugsch, T.: Spezielle Pathologie und Therapie innerer Krankheiten, Berlin, Urban & Schwarzenberg, 1913, vol. 2, pt. 2, p. 1164.

11. Jacob, L.: Ueber Allgemeininfektion durch Bacterium coli commune, Deutsches Arch. f. klin. Med. **97**:303, 1909.

12. This case was the subject of a paper read before the New York Pathological Society in April, 1931.

The patient's temperature rose to 106 F. on November 10. On November 15, an external urethrotomy with incision and drainage of the prostatic abscess was performed. Several drachms of pus were removed. No culture was taken. The patient was irrational but had no signs of increased intracranial pressure or meningeal irritation. A blood culture on November 17 was positive; a gram-negative bacillus was isolated which was later identified as Bact. acidi-lactici. A blood culture on November 26 was sterile. On November 29 the temperature indicated sepsis. A blood culture on December 2 was positive; the micro-organism was reported to be a bacillus in the Friedländer group, but not a true B. mucosus-capsulatus. On December 3 a physical examination revealed dulness at the base of the right lung, with râles in the base of the left lung. On December 4, broncho-vesicular breathing was present in the left side in addition to the râles. A loud systolic and a soft diastolic murmur was heard for the first time. The lung signs continued changing from day to day. On December 7, petechiae were found in the left conjunctiva, and the fingers on the right hand were painful. The murmurs were more pronounced. At this time the diagnosis of bacterial endocarditis was made, although the micro-organism obtained in blood cultures was not as yet accurately identified. Petechiae appeared elsewhere; in addition, the right wrist became painful and swollen. On December 10 the patient was slightly irrational. On December 11 he was exceedingly irrational and developed meningismus in the afternoon. That evening he suddenly became livid, and his pulse rate dropped to 48. He died thirty-five days after admission and twenty-seven days after the operation. The clinical diagnoses were subacute bacterial endocarditis, due to Bact. acidi-lactici, and abscess of the prostate.

Autopsy.—Autopsy was performed nine and a half hours post mortem. There were many petechiae in both conjunctivae and in the skin of the trunk and upper extremities. The perineal incision was clean and not draining. The most striking observations were on the heart, although most of the other organs showed changes.

The heart weighed 500 Gm. The auricles were covered with thick, gray, firmly adherent membrane. The right auricle was normal. The tricuspid valve was thin and delicate. At the base of the septal leaflet was a nodule about 0.5 cm. in diameter. On section an abscess was found in the wall of the auricle, surrounded by a hemorrhagic area. This extended to the base of the valve. The right ventricle was dilated and hypertrophied. The pulmonic valve was normal. In the left auricle there was an elevated hemorrhagic area in the fossa ovalis. The mitral valve was slightly thickened at the line of closure. Several small hemorrhagic areas were present in the base of the valve. A hemorrhagic area, about 3 mm. in diameter, was present in the posterior papillary muscle. The left ventricle was hypertrophied and dilated. The aortic valve leaflets were covered with vegetations which were soft, crumbly and pale yellow. The largest vegetation was 2.5 cm. in length and 1.5 cm. in thickness, and entirely covered the right posterior leaflet. This leaflet was ulcerated, leaving an opening about 2 mm. in diameter. Extending from this were small masses growing down over the endocardium of the left ventricle and the aortic leaflet of the mitral valve, and to the base of the tricuspid valve through the muscle. The areas to which the vegetations were adherent were hemorrhagic. The myocardium was firm, with no visible fibrosis. The coronary arteries were mildly sclerotic.

The aorta was moderately sclerotic.

The lungs were voluminous and covered with smooth pleura. Each weighed 840 Gm. They were crepitant throughout.

The spleen was enlarged and soft. On section the pulp bulged over the capsule.

The liver was normal.

The gallbladder was large and filled with thick greenish-black bile. Two stones, each about 1.5 cm. in diameter, were present. The gall bladder wall was thickened. The pancreas and suprarenals were normal.

The kidneys each weighed 260 Gm. The capsule of the right kidney stripped easily, leaving a very finely granular surface, which was pale yellowish-green with many small light yellow areas. On section the color was yellowish brown. The cortex was 8 mm. in thickness. Small abscess cavities filled with yellowish pus were present. Many tubules were seen in the pyramids as yellowish stripes. The left kidney had a more granular surface. The color was yellowish-brown with

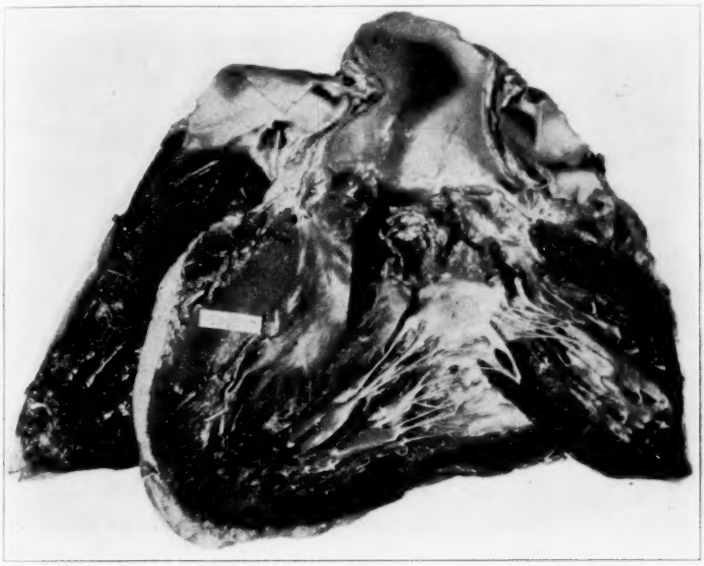

Fig. 1.—Acute bacterial endocarditis of the aortic valve (Bact. acidi-lactici).

small elevated yellowish areas. On section the color was pale reddish brown. The cortex was thin, measuring 5 mm. in thickness. Red longitudinal stripes were present. The pyramids were made up of alternate stripes of red and yellow. In them were several large abscess cavities up to 1.5 cm. in diameter, filled with pus. The bladder was thick-walled. The prostate was firm and asymmetric, and not enlarged. The left side was larger. The capsule was indistinct and merged with the periprostatic tissue. Many small pus-filled abscesses were present. The seminal vesicles and testes showed no changes. The gastro-intestinal tract was normal.

The brain weighed 1,450 Gm. Externally there was a hemorrhagic area on the parietal lobe of the right cerebral hemisphere close to the midline. On section many small pale red areas were found scattered through the cerebral hemispheres, internal capsules, cerebellar hemispheres and medulla. The spinal cord was not removed.

Bact. acidi-lactici was obtained in pure culture from the heart's blood.

Microscopic Examination: Sections of the heart, kidney and prostate showed the most marked changes.

In the myocardium were areas of polymorphonuclear infiltration and atrophy. Bacterial plugs filled many of the blood vessels. These were made up of short, plump rods which stained deep blue in the hematoxylin and eosin and methylthionine chloride, U. S. P. (methylene blue), sections and red in the Gram stains. In a section of the posterior papillary muscle atrophied muscle fibers were found in the polymorphonuclear-infiltrated reticulum. Hyaline material replaced part of the myocardium. Necrotic muscle fibers were present in these masses. A section of the myocardium taken from a point near the aortic valve showed thickening of the endocardium and subendocardial layers. Part of these structures were infiltrated

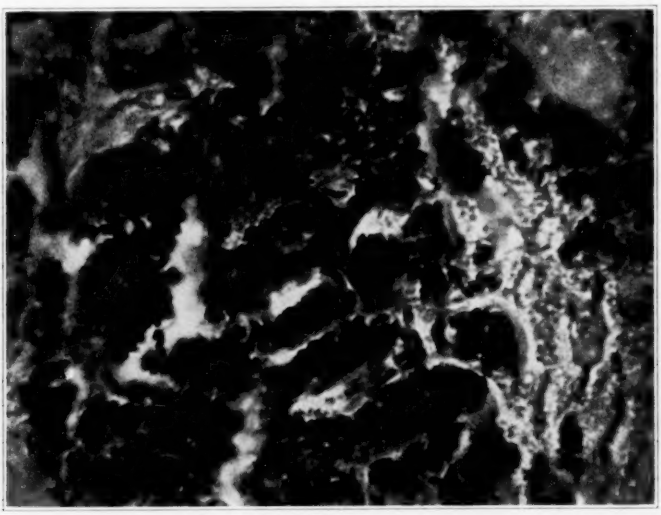

Fig. 2.—Photomicrograph, taken with the oil-immersion lens, of the vegetation on the aortic valve. Bacterial colonies, composed of large gram-negative bacilli, are seen.

with red blood cells and polymorphonuclear leukocytes, and then were replaced by a mass of fibrin containing many polymorphonuclear leukocytes, bacilli and necrotic débris. The necrosis extended into the myocardium, with the formation of an abscess. The muscle fibers around this area were necrotic and disintegrated. A methylthionine chloride section showed the presence of many bacilli.

The entire aortic valve leaflet taken for microscopic study was necrotic. The surface was covered with vegetations containing large masses of bacteria, polymorphonuclear leukocytes and nuclear débris. The micro-organism was found to be a gram-negative bacillus.

The aorta showed the changes of moderate arteriosclerosis.

The lung sections were normal.

In the liver sections, many polymorphonuclear leukocytes were found which were most numerous in the periportal fields. In many areas masses of bacteria were also present.

The gallbladder was thick-walled. The submucous layer was congested and infiltrated with plasma cells.

In the pancreas the polymorphonuclear leukocytes were increased in number in a few areas where the acini were shrunken or disintegrated.

The amount of lipoid material in the cortex of the suprarenals was diminished. Blue-staining bacterial plugs were found in several blood vessels.

Several sections of the kidney examined contained abscess cavities of varying sizes in the cortex and medulla. Areas of round cell infiltration were present. Bacterial plugs were found in several glomerular tufts. Bacilli could be seen throughout the section in the methylthionine chloride preparation.

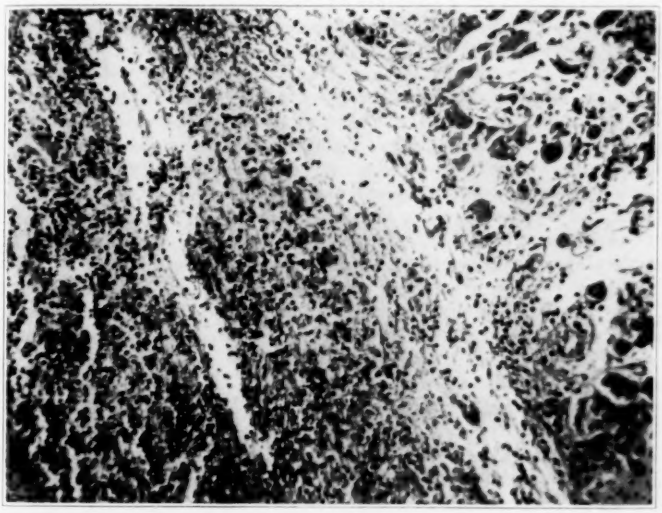

Fig. 3.—Photomicrograph, taken with the low power objective, of abscess in the myocardium of the interventricular septum. There is a dense infiltration of polymorphonuclear leukocytes and destruction of the muscle fibers.

The prostate showed variations in the size of the acini, which contained desquamated cells and débris. In a few, necrotic material and polymorphonuclear leukocytes were found. The stroma was infiltrated with round cells. A portion of the bladder which was present was infiltrated with round cells. In another section the prostatic tissue had undergone necrosis. Many polymorphonuclear leukocytes and necrotic débris were found in these areas. Dense round and plasma cell infiltrations were present in the stroma. Many bacilli were seen in the abscesses in the methylthionine chloride sections.

The tubules of the testes had thickened basement membranes. Several blood vessels were present whose walls and surrounding tissue were infiltrated with polymorphonuclear leukocytes and round and plasma cells.

The pituitary gland was normal. Several sections of the cerebrum were examined. In one localized area the meninges were infiltrated with many poly-

morphonuclear leukocytes and round cells. Bacterial plugs occluded several blood vessels in the substance of the cerebrum. About these vessels were found polymorphonuclear leukocytes and round cells, which for the most part were sharply perivascular; in only one area was the brain itself involved. In the basal ganglions and cerebellum were found areas of slight perivascular infiltration of round cells.

The anatomic diagnosis was: chronic and acute prostatitis; abscess of the prostate (external urethrotomy; perineal incision and drainage of prostatic abcess); cystitis cystica; acute bacterial endocarditis (Bact. acidi-lactici) of the aortic valve, with extension to the mitral and tricuspid valves; bacteremia (Bact. acidi-lactici) with metastases in the myocardium, pancreas, liver, kidneys, testes, brain and leptomeninges; acute splenic tumor; chronic cholecystitis, and cholelithiasis, with calculi in the gallbladder.

Case 2.—The patient, E. F., was admitted on May 10, 1931, to the genito-urinary service, Dr. J. B. Squier attending. Her complaints were chills, fever and nausea. About eighteen months before admission she had an attack which resembled the present one. Seven weeks before admission she had mild urinary symptoms accompanied by a low grade pyrexia. She apparently recovered completely in four weeks. For a period of two weeks she was well and able to get about. About six days before admission there occurred a relapse. She began to have daily chills and a rise of temperature to 105 F. She had nausea and some loss of weight, but no other gastro-intestinal or gallbladder symptoms. There was no pain during this time. Her urinary symptoms were very mild. There was no frequency, dysuria or hematuria. Because of the nausea her water intake was small. She was incontinent and irrational the day before admission. The temperature was 101.5 F., the pulse rate 128, respirations 28 and the blood pressure 154 systolic and 76 diastolic. She was a woman 63 years old who appeared acutely ill and restless but rational. Bronchial râles and slight changes in the breath sounds were noted in the lung examination. Examination of the heart revealed that it was overactive and enlarged; the sounds were clear. A loud systolic murmur was heard at the apex, which was transmitted to the precordium and axilla. Both kidneys were palpable; the right was larger and movable.

The following laboratory observations were made. The blood count showed: red cells 4,050,000, hemoglobin 90 per cent, white cells 36,000 and polymorphonuclear leukocytes 92 per cent. Urinalysis revealed: albumin + and sugar + (once in four examinations), many white blood corpuscles and a few red blood corpuscles. A blood culture showed Bact. acidi-lactici. A roentgenogram revealed an enlarged heart.

The patient had daily chills followed by a marked rise in temperature. On May 10, the peak was 103 F. On May 11 there were two episodes. The peaks were 104 and 106 F. On May 12, the temperature reached 104.5 F. A physical examination revealed that the heart was slightly larger than on admission, with a marked heaving apical impulse. A systolic murmur replaced the final sound. The blood culture showed several hundred colonies of Bact. acidi-lactici on the plate. Petechiae were observed for the first time. On May 13, the temperature reached 105 F. after a severe chill. This was followed by profuse diaphoresis and coma. Several new petechiae appeared. Signs of bronchopneumonia were noted in the right upper and left lower lobes. The pulse rate and temperature continued increasing, the latter reaching 107 F. at the time of exitus.

Autopsy.—The body was that of a rather obese white woman. A fading petechia was seen in the left conjunctival sac. The heart weighed 350 Gm. A large milky plaque was present over the anterior surface at the base of the right ventricle. The

right auricle was slightly dilated. The tricuspid leaflets were slightly thickened near the free margin. The right ventricle was normal, as were the pulmonic leaflets. The left auricle was slightly dilated. The mitral leaflets presented several areas of thickening near the free margin, but were competent. The papillary muscles were pale. The left ventricle was slightly dilated. On the right anterior leaflet of the aortic valve was an irregular grayish-red vegetation measuring about 1 cm. in its longest diameter. It felt fairly firm and projected toward the ventricular cavity. The other two leaflets of the aortic valve were normal. The myocardium felt fairly soft. The coronary vessels showed numerous grayish-yellow subintimal thickenings. The aorta showed changes of moderate arteriosclerosis. In the lungs, a few firm areas were felt in the lower lobes; otherwise they were normal. The spleen was soft, and on section the pulp overflowed the cut surface. The malpighian corpuscles were not distinct. The liver had a distinct yellowish tinge. The lobulations were distinct in some areas and obscure in others. The gallbladder was considerably distended, and was filled with about 35 cc. of pale greenish-brown turbid fluid which was not viscous. Within the gallbladder were four small faceted stones measuring about 1 cm. in their longest diameter. A similar stone was found in the cystic duct. The hepatic and common bile ducts were patent. The mucosa of the gallbladder over large areas was gangrenous and necrotic, and in parts separated from the underlying muscular wall. To the external surface of the fundus of the gallbladder the second portion of the duodenum was firmly adherent. The pancreas was normal. The surface of the kidneys was fairly smooth. Several depressed, irregular scars were present. The surface was yellowish gray. On the left kidney was an irregular reddish area about 1 cm. in diameter. On section, this extended into the cortex for about 8 mm. and was surrounded by a red zone, 2 mm. thick. Otherwise the cortex and medulla of both kidneys were normal. The pelvis and ureters were slightly injected. The bladder and genital organs were normal. There were numerous small punctate hemorrhages beneath the mucosa of the stomach. The duodenum was firmly adherent to the gallbladder. The remainder of the intestinal tract was normal. The brain and spinal cord were not removed. Bacteriologic Report: Bact. acidi-lactici was isolated from the heart's blood and Bact. acidi-lactici and Bact. coli were isolated from the gallbladder.

Microscopic Examination: The myocardium showed no changes. On the aortic valve was a large, irregular vegetation, consisting mostly of pinkish-staining, irregular strands of fibrin. On the surface were large masses of blue-staining bacteria. Near the surface between the fibrinous strands were dense accumulations of poorly preserved erythrocytes and masses of polymorphonuclear leukocytes. The ground substance of the valve was slightly edematous and densely infiltrated with polymorphonuclear leukocytes. Near the tip of the valve was a small isolated vegetation consisting of closely packed, blue-staining bacteria. At the base of the valve were found rather large, irregular masses of bacteria, and polymorphonuclear cells. The inflammatory reaction extended for only a short distance along the ventricular endocardium as well as the aortic intima. At the base of the aorta, the intima was markedly thickened and stained palely, containing only a few nuclei. The remainder of the wall consisted of rather dense collagenous fibers, slightly separated from each other. Between them were a few spindle-shaped nuclei. A section stained by Gram's method showed many short, plump, pink-staining rods. In the aorta were found changes of moderate arteriosclerosis. No exudate was found in the alveoli of the lungs. In one area were several large masses of closely packed bacteria. Some of the alveolar walls were densely infiltered with polymorphonuclear cells. The malpighian corpuscles of the spleen were small. The pulp contained a large

number of red blood cells, small mononuclears and a moderate number of polymorphonuclear cells. Bacterial colonies were also seen. The liver cells were large and stained poorly. Many small and large fat droplets were seen within them, throughout the lobule. At one point beneath the capsule were several large masses of densely packed bacteria. The liver cells about these masses were small and irregular, and stained poorly. There was, however, no polymorphonuclear reaction. The pancreas and suprarenals were normal. In the latter in a group of small vessels were found closely packed masses of bacteria. In the kidney, numerous glomeruli were completely hyalinized. The capsules of some of the glomeruli were thickened. The cells of the tubules were large, and stained palely. Between the tubules, numerous mononuclear cells were seen. In one of the collecting tubules was a large mass of bacteria. The interstitial tissue near it was densely infiltrated with small mononuclear cells. At one point there was rather extensive hemorrhage with necrosis of the tubules in the adjacent areas. In one section, a rather large infarct was present. At one point were several masses of bacteria surrounded by a collection of polymorphonuclear cells. The tubules in the adjacent areas appeared necrotic, and were separated from each other by edema and hemorrhage. The sections of the pelvic organs were normal. The section of the duodenum and gallbladder showed that the epithelial lining of the duodenum was missing. Brunner's glands were normal. The lymphatic vessels of the submucosa were distended, and within the muscular coats were numerous large collections of small lymphocytes. Binding the wall of the duodenum to the external wall of the gallbladder was a very dense band of connective tissue. In the latter were numerous lymphatics dilated with plugs of bacteria. Within it also were several accumulations of small mononuclear cells. The muscle layers of the gallbladder were separated by edematous newly formed connective tissue as well as by an infiltration of small mononuclear cells. The submucosa of the gallbladder was slightly edematous. At some points there was extensive hemorrhage, edema and necrosis of tissue. The lining epithelial cells were not present.

The anatomic diagnosis was: cholelithiasis, with calculi in the gallbladder and cystic duct; acute gangrenous cholecystitis (Bact. acidi-lactici); acute endocarditis of the aortic valve (Bact. acidi-lactici); bacteremia (Bact. acidi-lactici); infarct of the left kidney; acute splenic tumor; a fatty liver, and fibrous peritoneal adhesions.

COMMENT

Jacob,[11] in his nine cases of Bact. coli sepsis, found the original focus to be in the biliary passages, the urinary tract, the gastro-intestinal tract or the female genital tract. In forty-nine cases, Brian[13] found the biliary tract to be the focus in sixteen instances, the urinary tract in thirteen, the intestinal tract in twelve, and the female genital tract in nine. Felty and Keefer[14] reported the urinary tract to be the portal of entry in sixteen cases of twenty-eight; twelve of these occurred in males. They had no case originating in the biliary tract. They also

13. Brian, O.: Ueber Allgemeininfektion durch Bacterium coli commune (Colisepsis), Deutsches Arch. f. klin. Med. **106**:379, 1912.

14. Felty, A. R., and Keefer, C. S.: Bacillus Coli Sepsis: Clinical Study of Twenty-Eight Cases of Blood Stream Infection by Colon Bacillus, J. A. M. A. **82**:1430 (May 3) 1924.

found that the colon bacillus does not tend to invade the blood spontaneously, but usually follows cystoscopy or surgical trauma. This happened in twenty of the twenty-eight cases. When sepsis occurs, the local lesion is usually extensive. Libman[15] stated that "acute bacterial endocarditis arises secondarily to active purulent foci." In his experience, the colon bacillus enters the blood by way of the genito-urinary tract, uterus or gallbladder. The bacteremia or pyelonephritis due to Bact. coli or Bact. mucosum-capsulatum follows genito-urinary manipulation.

The original focus of infection in case 1 was most probably the prostate gland. The biliary tract can be safely ruled out as the original source, since the lesions in the gallbladder were chronic in character, and there were no active lesions. The focus in the second case, however, was definitely in the gallbladder. The points of similarity between these cases and those of Bact. coli sepsis can be brought out by comparison.

In the first case, the patient showed no signs of heart involvement when he entered the hospital. Following the opening of the abscess there was invasion of the blood stream by the organism since the blood culture showed the presence of a gram-negative bacillus two days later. The highest leukocyte count was obtained at this time. His temperature began to drop after this, and nine days later the blood culture was sterile. His temperature then indicated renewal of the sepsis, and a heart murmur was heard which became louder from day to day. The blood culture again showed the presence of Bact. acidi-lactici. This may well be interpreted as indicating the invasion of the blood following surgical trauma, with localization of the bacilli on the aortic valve leaflets.

Repeated blood cultures after this showed the presence of Bact. acidi-lactici. The final changes were due to detachment of tiny bits of vegetation which gave rise to the petechiae in the skin, the miliary purulent foci in the viscera and the bacterial plugs in the vessels. The second peak of leukocytosis was obtained at this time.

In the second case, the focus was undoubtedly in the gallbladder. Though the history is inadequate, the "attack" that was described as occurring eighteen months before admission might well have been one of acute cholecystitis, superimposed on chronic cholecystitis with stone formation. The causative organism is not known. This acute attack subsided and healed, with the formation of adhesions between the gallbladder and duodenum. Another acute attack occurred shortly before admission. The causative organism again is not known. It is possible that the Bact. acidi-lactici reached the gallbladder from the loop of duodenum which was adherent, or that it was the primary cause of the infection. The establishment of a purulent focus in the gallbladder was

15. Libman, E.: General Infections by Bacteria, J. Michigan M. Soc. **23**:462, 1924.

followed by sepsis and the coincident infection of the aortic valve. With the subsidence of the sepsis, which is usually of short duration,[13] there was a period of two weeks during which the patient was symptom-free. Following the formation of the vegetation, sepsis was renewed and the valve became the important source for dissemination of the organism, because of the emboli that became detached.

There is one point of resemblance in these two cases of Bact. acidi-lactici endocarditis that may be important or merely a coincidence. The aortic valve leaflets were the site of the vegetation formation. A single case of Bact. coli sepsis with endocarditis in the Presbyterian Hospital records also shows involvement of the aortic valve. The case of Bact. coli endocarditis reported by Horder[7] showed at autopsy large, lumpy, friable, easily detached vegetations on the aortic valve.

SUMMARY

Two cases of Bact. acidi-lactici endocarditis, closely resembling cases of infection with Bact. coli, are reported because of their rarity. More complete bacteriologic studies of cases of Bact. coli sepsis will undoubtedly bring to light more examples of Bact. acidi-lactici infections. The coincidence of aortic valve involvement in two cases of Bact. acidi-lactici endoarditis and in two cases of Bact. coli endocarditis is pointed out.

EFFECT OF HISTAMINE ON ALKALI RESERVE AND ON BLOOD SUGAR IN MAN

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Histamine, β-imidazolylethylamine, is a decomposition product of albumin and is derived from the amino-acid histidine by the splitting off of carbon dioxide. It is exceedingly active both pharmacologically and physiologically, and has therefore been widely studied.

The action of histamine on the secretory function of the stomach was proved in animal experimentation by Popielski,[1] Keeton, Koch and Luckhardt[2] and others. On the human stomach its effect was first observed in 1922 by Carnot, Koskowski and Liebert,[3] and since that time it has been the subject of numerous investigations. Matheson and Ammon,[4] Gompertz and Vorhaus,[5] Andresen,[6] Bockus and Bank,[7]

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2. Keeton, R. W.; Koch, F. C., and Luckhardt, A. B.: Gastrin Studies: III. The Response of the Stomach Mucosa of Various Animals to Gastrin Bodies, Am. J. Physiol. **51**:454, 1920.

3. Carnot, P.; Koskowski, W., and Liebert, E.: L'influence de l'histamine sur la sécrétion des sucs digestifs chez l'homme, Compt. rend. Soc. de biol. **86**:575, 1922.

4. Matheson, A. K., and Ammon, S. E.: Observations on the Effect of Histamine on the Human Gastric Secretion, Lancet **1**:482, 1923.

5. Gompertz, L. M., and Vorhaus, M. G.: Studies on the Action of Histamine on Human Gastric Secretion, J. Lab. & Clin. Med. **11**:14, 1925.

6. Andresen, A. F. R.: Fractional Gastric Analysis with Histamine, Tr. Am. Gastro-Enterol. A., 1926, p. 53.

7. Bockus, H. L., and Bank, J.: The Value of Histamine as a Test for Gastric Function, Arch. Int. Med. **39**:508 (April) 1927.

Katzenelbogen and Choisy,[8] Bloomfield and Polland,[9] Fényes[10] and Henning[11] demonstrated that histamine injected either subcutaneously or intramuscularly is a most reliable stimulant for gastric secretion, especially the secretion of free hydrochloric acid, and that the secretion of hydrochloric acid occurs within fifteen minutes after the injection of histamine, usually reaching a maximum in about from thirty to sixty minutes.

Because of the increase in the acidity of the stomach secretion, Katzenelbogen[12] reasoned that a corresponding general decrease of acidity should take place in all other body fluids. This hypothesis was apparently confirmed at least in part by his experimental study on rabbits. He observed that sixty minutes after the intramuscular injection of 1 cc. of a 1:1,000 histamine solution there was an increase of the alkali reserve from 22.2 to 105 per cent, or an average of 50.55 per cent. On the basis of these results and on the assumption that histamine increases the alkali reserve by draining acidity into the stomach, he suggested that it might be of value in the treatment for nondiabetic acidosis, especially if combined with the parallel elimination of the acidity either by the removal of the stomach contents or by neutralization with alkali given by mouth. As to its use in diabetic acidosis and coma, he cautioned that histamine, as he and Abramson[13] and La Barre[14] showed, provokes hyperglycemia. On the other hand, Hiller[15] studied the effect of the subcutaneous injection of histamine on the acid-base balance of normal dogs. The dose varied from 0.1 to 3 mg. per kilogram of body weight. She found that from forty to ninety minutes after the injection of the histamine, the p_H of the blood and the bicarbonate of the plasma were both decreased. Corresponding with this change there was an increased excretion of alkali in the urine.

8. Katzenelbogen, S., and Choisy, R.: L'influence de l'histamine sur la sécrétion gastrique, Arch. de mal. de l'app. digestif **17**:278, 1927; Étude de la sécrétion gastrique par l'épreuve de l'histamine, Schweiz. med. Wchnschr. **57**:1009, 1927.

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13. Katzenelbogen, S., and Abramson, A.: Les variations du taux de la glycémie consécutives aux injections intra-veineuse et intra-cardiaque d'histamine, Compt. rend. Soc. de biol. **97**:240, 1927.

14. La Barre, Jean: À propos des variations de la glycémie consécutives à l'injection intraveineuse d'histamine, Compt. rend. Soc. de biol. **94**:779, 1926.

15. Hiller, A.: The Effect of Histamine on the Acid-Base Balance, J. Biol. Chem. **68**:833, 1926.

Boyd, Tweedy and Austin [16] injected 0.7 mg. of histamine subcutaneously into dogs weighing from 9 to 12 Kg. and observed a rise in the blood p_H amounting to from 0.02 to 0.06, from one to two hours after the injection. With larger doses a decrease in p_H was observed.

TABLE 1.—*Carbon Dioxide Combining Power of Plasma; Control Experiments*

Patient	Time Between Blood Samples, Minutes	Alkali Reserve: First Blood Sample, per Cent by Volume	Alkali Reserve: Second Blood Sample, per Cent by Volume	Difference, per Cent by Volume
1	45	56.5	55.5	−1.0
2	45	54.8	53.0	−1.8
3	50	60.2	60.2	None
4	55	57.7	55.8	−1.9
5	60	62.3	59.5	−2.8
6	60	59.3	58.9	+0.4
7	65	54.7	55.1	+0.4
8	65	60.9	61.0	+0.1
9	70	60.4	57.9	−2.5
Average difference				−1.1

TABLE 2.—*Carbon Dioxide Combining Power of Plasma Before and After the Injection of 0.5 Mg. of Histamine*

Patient	Time Between Blood Samples, Minutes	Alkali Reserve: First Blood Sample, per Cent by Volume	Alkali Reserve: Second Blood Sample, per Cent by Volume	Difference, per Cent by Volume
1	20	66.6	60.0	−6.6
2	25	61.9	61.9	±0.0
3	35	58.5	64.2	+5.7
4	35	68.8	68.8	±0.0
5	40	57.6	65.2	+7.6
6	40	59.0	63.1	+4.1
7	45	56.8	60.5	+3.7
8	45	59.3	63.7	+4.4
9	45	64.1	69.1	+5.0
10	45	63.5	67.5	+4.0
11	50	57.4	57.9	+0.2
12	50	55.5	58.2	+2.7
13	55	60.5	55.3	−5.2
14	60	71.4	68.7	−2.7
15	60	67.8	68.6	+0.8
16	60	54.1	61.6	+7.5
17	60	63.2	65.0	+1.8
18	60	56.5	56.5	±0.0
19	75	71.3	73.2	+1.9
Average difference				+1.8

Because the response of the acid-base balance has been found to vary with (1) the amount of histamine injected, (2) the manner in which it is administered and (3) the species in which the observations are made, we decided to investigate whether a change occurs in the

16. Boyd, T. E.; Tweedy, W. R., and Austin, W. C.: Some Effects of Histamine on the Acid-Base Balance, Proc. Soc. Exper. Biol. & Med. **25**:451, 1928.

alkali reserve in man when the dose corresponds to that employed clinically, namely, from 0.5 to 0.75 mg.

Further, since Katzenelbogen and Abramson [13] working with guinea-pigs, Ni [17] working with dogs and Fényes [10] working with human subjects had shown that histamine produces hyperglycemia, it was also decided to investigate its effect on the blood sugar level in man.

Our observations were carried out on ward patients in Prof. Alfred Luger's Medical Abteilung of the Kaiserin Elisabeth Hospital in Vienna. Most of the subjects were young adults; all were free from fever and without an obvious disturbance of gastric function.

TABLE 3.—*Carbon Dioxide Combining Power of Plasma Before and After the Injection of 0.75 Mg. of Histamine*

Patient	Time Between Blood Samples, Minutes	Alkali Reserve: First Blood Sample, per Cent by Volume	Alkali Reserve: Second Blood Sample, per Cent by Volume	Alkali Reserve: Difference, per Cent by Volume
1	30	61.3	60.1	−1.2
2	35	64.3	67.6	+3.3
3	35	59.8	62.1	+2.3
4	40	57.4	54.3	−3.1
5	45	67.7	68.7	+1.0
6	50	62.3	62.3	None
7	50	64.6	64.1	−0.5
8	50	64.5	64.7	+0.2
9	50	60.8	62.8	+2.0
10	50	69.3	69.3	None
11	50	60.8	61.8	+1.0
12	55	60.7	71.2	+10.5
13	55	52.2	45.4	−6.8
14	55	62.3	62.3	None
15	55	56.5	58.8	+2.3
16	60	60.0	57.7	−2.3
17	60	66.1	67.6	+1.5
18	75	56.2	55.9	−0.3
Average difference				+0.55

TECHNIC

All subjects were fasting and at rest in bed; they received no food or drink between the blood takings. The experiments were carried on between the hours of 8 and 10 a. m. In every case in which histamine was used, it was given in 1:1,000 solution so that 1 cc. contained 1 mg. Ten cubic centimeters of blood was taken from an arm vein into a tube containing a small amount (0.05 Gm.) of potassium oxalate and under paraffin oil before and from twenty to seventy-five minutes after the intramuscular or subcutaneous (gluteal) injection of histamine. The specimens were immediately centrifugated, after which approximately 3 cc. of plasma was pipetted off, placed in a separatory funnel and saturated with alveolar air. The plasma carbon dioxide combining power was then determined by the direct method of Van Slyke and Cullen. For the blood sugar determination, speci-

17. Ni, T. G.: On the Inverse Change Between the Concentration of Glucose and Chloride in the Blood, Am. J. Physiol. **78**:158, 1926.

mens were taken in duplicate from an arm vein at the same time as for the alkali reserve. The micromethod of Bang was employed, the average of the two determinations being given in the accompanying tables. Seventy patients were used for this study, and although one hundred cases are presented in our tables, this apparent discrepancy is due to the fact that parallel blood sugar and alkali reserve determinations were made in thirty cases.

RESULTS

In table 1 are presented nine cases used as controls. The figures show very little variation in the alkali reserve in from forty-five to

TABLE 4.—*Carbon Dioxide Combining Power of Plasma Before and Sixty Minutes After 2 Gm. of Sodium Bicarbonate by Mouth*

	Alkali Reserve		
Patient	First Blood Sample, per Cent by Volume	Second Blood Sample, per Cent by Volume	Difference, per Cent by Volume
1	60.0	60.6	+0.6
2	59.4	61.8	+2.4
3	64.1	66.5	+2.4
4	63.0	72.4	+9.4
5	59.9	69.1	+9.2
6	64.2	71.2	+7.0
Average increase			+5.17

TABLE 5.—*Carbon Dioxide Combining Power of Plasma Before and Sixty Minutes After 2 Gm. of Sodium Bicarbonate by Mouth and 0.75 Mg. of Histamine Subcutaneously*

	Alkali Reserve		
Patient	First Blood Sample, per Cent by Volume	Second Blood Sample, per Cent by Volume	Difference, per Cent by Volume
1	68.8	75.3	+6.5
2	66.7	71.6	+4.9
3	64.0	73.0	+9.0
4	64.0	71.8	+7.2
5	66.9	76.7	+9.8
6	68.2	73.9	+5.7
Average increase			+7.18

seventy minutes. The average variation is a decrease of 1.1 per cent by volume.

Table 2 shows the results in nineteen cases from twenty to seventy-five minutes after the intramuscular injection of 0.5 mg. of histamine. A small increase is noted in thirteen cases, a decrease in three and no change in three. The average variation was an increase of 1.8 per cent by volume.

In table 3 are shown the figures in eighteen cases after the intramuscular injection of 0.75 mg. of histamine. A small increase in the alkali reserve occurred in nine cases, and in one case an increase of

10.5 per cent by volume. There was a decrease in six cases and no change in three. The average was an increase of only 0.55 per cent by volume.

We then tried giving alkali by mouth in the form of sodium bicarbonate in an attempt thereby to neutralize hyperacidity of the gastric contents, and to prevent, if possible, the reabsorption of the acid. The results are seen in tables 4 and 5. The patients in the six cases in table

TABLE 6.—*Carbon Dioxide Combining Power of Plasma Before and After 2 Gm. of Tribasic Calcium Phosphate by Mouth*

	Alkali Reserve		
Patient	First Blood Sample, per Cent by Volume	Second Blood Sample, per Cent by Volume	Difference, per Cent by Volume
1	61.9	61.7	−0.2
2	63.7	63.7	None
3	61.2	62.1	+0.9
4	55.4	54.6	−0.8
5	51.6	51.6	None
6	59.1	61.0	+1.9
Average increase			+0.3

TABLE 7.—*Carbon Dioxide Combining Power of Plasma Before and After 2 Gm. of Tribasic Calcium Phosphate by Mouth and the Injection of 0.5 Mg. of Histamine Subcutaneously*

	Alkali Reserve		
Patient	First Blood Sample, per Cent by Volume	Second Blood Sample, per Cent by Volume	Difference, per Cent by Volume
1	58.4	61.9	+3.5
2	59.8	57.5	−2.3
3	68.7	73.1	+4.4
4	59.3	62.0	+2.7
5	61.2	65.9	+4.7
6	59.2	61.6	+2.4
Average increase			+2.6

4 used as controls were given 2 Gm. of sodium bicarbonate by mouth. In the second group (table 5) the six patients were given 2 Gm. of sodium bicarbonate and 0.5 mg. of histamine subcutaneously. An increase in the alkali reserve occurred in every case in both groups. The average increase in the controls was 5.17 per cent by volume, while the average in the group that received both alkali and histamine was somewhat greater, namely, 7.18 per cent by volume.

Mindful of the objection that might be raised that the increase in the alkali reserve was due to the absorption of the sodium bicarbonate, we decided to give tribasic calcium phosphate, which neutralizes the

acidity of the gastric secretion without producing an alkaline solution.[18] Six patients were given 2 Gm. of this alkali by mouth, and six received in addition 0.5 mg. of histamine subcutaneously. Tribasic calcium phosphate alone gave a small increase of 0.3 per cent by volume, while when combined with histamine the average increase was still only 2.6 per cent by volume.

In table 8 are listed the cases of six patients used as controls for blood sugar determinations. The variations range from an increase of

TABLE 8.—*Blood Sugar in Milligrams per Hundred Cubic Centimeters; Control Experiments*

Patient	Time Between Blood Samples, Minutes	First Blood Sample	Second Blood Sample	Difference
1	45	105.5	102.0	−3.5
2	45	107.0	100.0	−7.0
3	50	112.0	104.0	−8.0
4	55	97.0	98.5	+1.5
5	60	108.5	110.0	+1.5
6	50	104.0	101.0	−3.0
Average difference				−3.1

TABLE 9.—*Blood Sugar in Milligrams per Hundred Cubic Centimeters Before and After the Intramuscular Injection of 0.5 Mg. of Histamine*

Patient	Time Between Blood Samples, Minutes	First Blood Sample	Second Blood Sample	Difference
1	20	92.5	94.0	+1.5
2	25	118.5	115.5	−3.0
3	35	112.0	107.0	−5.0
4	35	107.0	114.0	+7.0
5	40	121.0	128.0	+7.0
6	40	116.0	124.0	+8.0
7	45	121.0	121.0	None
8	50	114.0	110.0	−4.0
9	50	121.0	119.5	−1.5
10	55	118.0	114.0	−4.0
11	60	107.0	107.0	None
12	75	120.0	120.0	None
Average difference				+0.50

1.5 mg. per hundred cubic centimeters to a fall of 8 mg., the average variation being a fall of 3 mg. in from forty-five to sixty minutes.

In table 9 are presented twelve cases, showing the blood sugar in milligrams per hundred cubic centimeters from twenty to seventy-five minutes after the intramuscular injection of 0.5 mg. of histamine. The results varied, with an increase in four cases, a decrease in five and no change in three, the average variation being an increase of 0.5 mg. per hundred cubic centimeters.

In table 10 are listed the results in twelve cases in from thirty to seventy-five minutes after the intramuscular injection of 0.75 mg. of

18. Hiller (footnote 15). Boyd, Tweedy and Austin (footnote 16).

histamine. As in table 9, no definite, uniform effect occurred. There was an increase in four cases, a fall in seven and no change in one. The average difference was an increase of 1.25 mg. per hundred cubic centimeters.

COMMENT

From our results it is evident that the intramuscular injection of histamine does not produce a uniform and significant increase either in the alkali reserve or in the blood sugar. While it is true that a small increase in the carbon dioxide combining power of the blood plasma did occur in most of the cases after the injection of histamine, it was not of sufficient degree to encourage the use of histamine as a therapeutic agent in acidosis. We are unable to explain why more cases showed an

TABLE 10.—*Blood Sugar in Milligrams per Hundred Cubic Centimeters Before and After the Intramuscular Injection of 0.75 Mg. of Histamine*

Patient	Time Between Blood Samples, Minutes	First Blood Sample	Second Blood Sample	Difference
1	30	100.0	97.0	−3.0
2	35	95.5	100.0	+1.5
3	35	104.0	112.0	+8.0
4	40	116.0	110.0	−6.0
5	45	15.5	89.0	−6.5
6	50	102.0	102.0	None
7	50	104.0	102.0	−2.0
8	50	107.0	105.5	−1.5
9	60	97.0	98.5	+1.5
10	60	110.0	105.5	−4.5
11	60	113.5	110.0	−3.5
12	75	99.5	101.5	+1.0
Average difference				+1.25

increase after the smaller dose of 0.5 mg. of histamine than after the injection of 0.75 mg.; the slight difference, however, is insignificant.

The combined administration of sodium bicarbonate by mouth and 0.75 mg. of histamine subcutaneously does produce a uniform and fairly significant increase in the alkali reserve. This increase is not significantly greater than that produced by sodium bicarbonate alone.

Tribasic calcium phosphate alone caused no appreciable increase in the alkali reserve; combined with the subcutaneous administration of histamine, a slight increase occurred.

That the pouring forth of hydrochloric acid in the stomach following the injection of histamine does not result in a decrease in the acidity of all other body fluids is now known. Matzner and Gray [19] have reported that histamine produces an increased secretion of bile and alkaline intestinal juices. Also they found that no more than 50 per

19. Matzner, M., and Gray, I.: The Comparative Changes in the Gastric Acidity and Urinary Reaction After the Injection of Histamine, Arch. Int. Med. **47**:202 (Feb.) 1931.

cent of the patients, in whose gastric contents free hydrochloric acid was demonstrated after the subcutaneous injection of histamine, had a definite alkaline tide in the urine. Further, Neale and Klumpp,[20] working with children of different ages, found that following the injection of histamine there occurred an increase in the alkalinity of the duodenal contents accompanying the secretion of acid in the stomach.

While a few cases showed a small increase in the blood sugar after the injection of histamine, the average change was slight.

CONCLUSIONS

1. The intramuscular injection of from 0.5 to 0.75 mg. of histamine usually produces a small increase in the alkaline reserve in man. The increase, however, is not sufficient to warrant its therapeutic application in acidosis.

2. The combined administration of sodium bicarbonate by mouth and histamine subcutaneously produces a significant increase in the alkali reserve, but the increase is not significantly greater than that produced by the alkali alone. Tribasic calcium phosphate alone or combined with histamine increases the alkali reserve only slightly.

3. The intramuscular injection of from 0.5 to 0.75 mg. of histamine does not produce a significant increase in the blood sugar level in man.

Prof. Alfred Luger gave many helpful suggestions and permitted us to use his cases in the Kaiserin Elisabeth Spital, Vienna, Austria.

20. Neale, A. V., and Klumpp, T. G.: Action of Histamine on the Pancreas, J. Clin. Investigation **9**:197, 1930.

EFFECT OF QUINIDINE SULPHATE IN INHIBITING VENTRICULAR FIBRILLATION

AN EXPERIMENTAL STUDY

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It has been quite definitely established that quinidine prolongs the refractory period of heart muscle; this has been regarded as the mechanism by which quinidine stops conditions due to circus movement of the heart such as auricular flutter and fibrillation. From clinical experience it has also been known that repeated administration of quinidine by mouth prevents the inception of these disturbances in the auricle which otherwise would be recurring in transient form.[1] Because fibrillation of the ventricles is also regarded as due to a circus movement, a similar method of therapy might become available as a preventive measure. Morawitz,[2] on empiric grounds, successfully used the drug as a prophylactic agent in patients whom he regarded as liable to sudden cardiac death; his figures showed a distinct diminution in the number of sudden deaths under the quinidine regimen. It is the general opinion at the present time that ventricular fibrillation is responsible for many cases of sudden cardiac death.[3] Stepp and Parade[4] and Weber[5] likewise advocated cautious quinidine medication, which they believe prevents or postpones the sudden fatal outcome.

On the other hand, some investigators reported that quinidine may tend to favor the inception of ventricular fibrillation. Kerr and Bender[6]

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From the Laboratories of Physiology in the Harvard Medical School.

1. Bramwell, J. C., and Ellis, R.: The Ultimate Results of Quinidine Therapy in Auricular Fibrillation, Lancet **2**:960 (Nov. 10) 1928.

2. Morawitz, P., and Hochrein, M.: Zur Verhütung des akuten Herztodes, München. med. Wchnschr. **76**:1075, 1929.

3. Hering, H. E.: Der Sekundenherztod, Berlin, Julius Springer, 1917.

4. Stepp, W., and Parade, G. W.: Untersuchungen und Betrachtungen über den plötzlichen Herztod durch Kammer-Flimmern, München. med. Wchnschr. **75**: 1869, 1928.

5. Weber, A.: Ueber den plötzlichen Herztod, Klin. Wchnschr. **6**:2458, 1927. (See also footnote 19.)

6. Kerr, W. J., and Bender, W. L.: Paroxysmal Ventricular Fibrillation with Cardiac Recovery in a Case of Auricular Fibrillation and Complete Heart Block While Under Quinidine Sulphate Therapy, Heart **9**:269, 1922. (See also footnote 18.)

reported the occurrence of paroxysmal ventricular fibrillation with recovery, in a case of auricular fibrillation and complete heart block, during quinidine sulphate therapy. They raise the question whether the quinidine was responsible for the ventricular fibrillation. Drury, Horsfall and Munly [7] found that in dogs ventricular fibrillation was occasionally produced by quinidine. They attributed these anomalous results either to the mode of stimulation employed (slow rhythmic shocks rather than the faradic current) or to the low degree of poisoning produced by the drug. In experiments in which larger doses of quinidine were used and in which the faradic current was employed, they found that fibrillation was produced with difficulty if at all. It is suggested that a dose of the drug, which produces a greater effect in prolonging conduction time than in prolonging the refractory period, might well induce or perpetuate ventricular fibrillation rather than prevent or terminate it.

The purpose of the present research was to obtain definite experimental evidence regarding the effect of quinidine on the ease of elicitation of ventricular fibrillation, and establish a rational basis for its use as a preventive of such a disturbance. Drury, Horsfall and Munly [7] showed that quinidine has the same effect on ventricular muscle that it has on heart muscle as a whole and on auricular muscle, that is to say, it lengthens the absolute refractory period and reduces the rate of conduction. Hecht and Rothberger [8] found that under quinine the ventricle of the cat's heart is thrown into fibrillation with difficulty or not at all; they made no similar study of quinidine.

EXPERIMENTAL METHOD

Since the cat's heart presents the advantage of recovering spontaneously from ventricular fibrillation,[9] cats were used in these experiments. The cat's heart can be stimulated repeatedly, and repeated determination of a "threshold" current for fibrillation can be made.

In the first experiments ethyl carbamate (urethane) anesthesia was employed. In the later series the animals were decerebrated by the method described by Cannon and Britton.[10] A stylet is driven swiftly through the thin petrous portion

7. Drury, A. N.; Horsfall, W. N., and Munly, W. C.: Observations Relating to the Action of Quinidine Upon the Dog's Heart: The Refractory Period of, and Conduction in, Ventricular Muscle, Heart **9**:365, 1922.

8. Hecht, A. V., and Rothberger, C. J.: Experimentelle Beiträge zur Kenntnis der Chininwirkung bei Herzflimmern, Ztschr. f. d. ges. exper. Med. **7**:134, 1919.

9. Porter, W. T.: The Recovery of the Heart from Fibrillary Contractions, Am. J. Physiol. **1**:71, 1898. d'Halluin, M.: Le massage du coeur, Presse méd. **12**:345, 1904. MacWilliam, J. A.: Fibrillar Contraction of the Heart, J. Physiol. **8**:296, 1887.

10. Cannon, W. B., and Britton, S. W.: Studies on the Conditions of Activity in Endocrine Glands: XV. Pseudaffective Medulliadrenal Secretion, Am. J. Physiol. **72**:286, 1925.

of the temporal bone about 1 cm. above the external auditory meatus with a quick blow of a hammer (no anesthesia having been administered), and on reaching the opposite bony wall, the tip of the stylet is immediately drawn across the floor of the skull with a single rapid stroke, thus disconnecting the cerebral peduncles from the brain stem. The animal at once goes into decerebrate rigidity and survives in this condition for several hours. The trachea is then cannulated and artificial respiration begun.

The heart is exposed in situ, and the pericardium sewed to the thoracic wall by a modification of the Drinker technic.[11] The heart can then be inspected directly. Fibrillation is produced by stimulating the heart directly with a bipolar stimulating electrode attached to the secondary coil of a large inductorium. The secondary coil is closely coupled to the primary and the current in the primary coil is varied by means of a rheostat; the current in the secondary coil varies directly with that in the primary and not logarithmically as it would if the current were varied by changing the distance between the two coils. An interruptor and an ammeter are included in the primary circuit. The heart is stimulated in the same spot each time; in the later experiments wire electrodes were sewed directly into the ventricular wall so that no variation in the site of stimulation was possible. The stimulation is applied for the same length of time on each occasion, five seconds unless otherwise stated. The cat is maintained at constant temperature and the exposed heart is kept moist with 0.9 per cent saline at body temperature.

The heart is stimulated with gradually increasing strengths of current, with intervals between stimulation adequate for the heart to recover, until the strength of current at which persistent fibrillation is produced has been determined. This current is called the threshold for ventricular fibrillation. At currents below this value the heart either is unaffected, is accelerated, or goes into fibrillation only while the current is being applied. This latter condition has been called "pseudofibrillation."[12] In true fibrillation a mechanism of circulating excitation is established, whereas in pseudofibrillation this is not so. With some exceptions stimulation with currents above the threshold value produces persistent fibrillation, while subthreshold currents cause, at the most, pseudofibrillation. In several experiments, however, it was found that currents above the strength which was first believed to be the "threshold" current, did not produce true fibrillation and, conversely, that currents below that value did produce persistent fibrillation, despite all efforts to preserve identical conditions. (Experiments in which it was impossible to check the thresholds by redetermination have been excluded from the tabulations). Most observers have not reported such anomalous results but apparently have been content to designate as the threshold the first strength of current at which fibrillation is produced, working up gradually from weaker currents. The present research seems to show that although this value generally is the true threshold it is not always so. The production of persistent fibrillation appears to be the result of changes in other variables than the amperage of the applied current, such as the site of stimulation,[13] the temperature of the heart,[14] exhaustion,[14] asphyxia, etc.

11. Drinker, C. K.: A Useful Heart Method, J. Exper. Med. **33**:675, 1921.

12. MacWilliam, J. A.: The Mechanism and Control of Fibrillation in the Mammalian Heart, Proc. Roy. Soc. London, s.B. **90**:302, 1917-1919.

13. Braun, L., and Samet, B.: Experimentelle Untersuchungen an den Koronargefässen und über Kammerflimmern. (Zur Prophylaxe des Flimmerns), Wien. klin. Wchnschr. **44**:136, 1931.

14. MacWilliam (footnote 9).

Once true fibrillation is produced, the heart may recover spontaneously after a so-called "postundulatory pause."[15] In the event that the heart does not recover of itself, remedial measures such as rhythmic compression of the ventricles (so-called "cardiac massage")[16] or the intracardiac injection of a very weak solution of epinephrine (0.1 cc. 1:10,000—1:5,000) may prove effective. When the depressed heart is massaged, the spontaneous beat develops more readily during a pause. One can count on successful resuscitation by massage only as long as the heart possesses a certain tone, that is, while there still remains a sense of firmness. Once the heart muscle is weak and flaccid, massage is of no avail. The resuscitatory efforts are not always successful, particularly with heavier cats, and many of the experiments ended with the determination of but one threshold.

EXPERIMENTAL RESULTS

In the first set of experiments the threshold current was first determined for the normal or unquinidinized animal; then the threshold

TABLE 1.—*Effect of the Absorption of Quinidine in Causing a Rise in the Threshold for Ventricular Fibrillation*

Exper. No.	Date	Weight, Kg.	Anesthesia	Threshold Before Quinidine	Threshold After Quinidine
1	2/18/30	2.3	Ethyl carbamate	11.77 cm. between coils	8.38 cm. between coils
2	4/ 9/30	2.3	Ethyl carbamate	0.30 amperes or less	0.43 amperes
3	7/22/30	2.3	Ethyl carbamate	0.25 amperes	0.35 amperes
4	7/23/30	2.8	Ethyl carbamate	0.35 amperes	0.40 amperes
5	7/24/30	3.5	Ethyl carbamate	0.20 amperes	0.30 amperes
6	7/25/30	2.7	Ethyl carbamate	0.35 amperes	0.55 amperes
7	7/28/30	1.7	Ethyl carbamate	0.30 amperes	1.50 amperes†
8	7/29/30	1.9	Ethyl carbamate	0.15 amperes	0.50 amperes
9	8/ 1/30	3.3	Ethyl carbamate	0.30 amperes	1.50 amperes
10	8/ 3/30	1.5	Ethyl carbamate	0.35 amperes	0.60 amperes or higher
11	9/25/30	1.9	Ethyl carbamate	0.05 amperes or less	1.50 amperes
12	10/30/30	1.6	Ethyl carbamate	0.10 amperes	0.15 amperes
13	11/24/30	1.8	Ethyl carbamate	0.15 amperes	0.30 amperes
14	2/10/31	2.4	Decerebrate	0.05 amperes or less	0.15 amperes
15	2/12/21	2.1	Decerebrate	0.10 amperes	0.15 amperes
16	2/26/31	2.9	Decerebrate	0.10 amperes	0.20 amperes
17	6/ 6/31	2.7	Decerebrate	0.20 amperes	1.50 amperes
18*	6/29/31	3.1	Decerebrate	0.10 amperes	0.30 amperes
	Average			0.20 amperes	0.60 amperes

* Heart denervated.
† Thirty seconds' duration.

was determined after the intravenous injection of an adequate dosage of quinidine sulphate. The second determination was made in from fifteen to thirty minutes after the injection, to give ample time for the quinidine to distribute itself in the heart muscle. The dosage used was from 5 to 10 mg. per kilogram of body weight of the cat. It did not seem to be material whether the smaller or larger dose was used.

In all the experiments of this series, excepting numbers 4, 12 and 15, quinidine sulphate made necessary an appreciably stronger faradic

15. Winterberg, H., in Handbuch der Normalen und Pathologischen Physiologie, Herzflimmern und Herzflattern: I. Blut Zirkulation-Herz, Berlin, Julius Springer, 1924, vol. 7, p. 663. MacWilliam (footnote 9).

16. Levy, A. G.: Massage of the Fibrillating Ventricles, Heart **7**:175, 1920. MacWilliam (footnote 9).

current in order to cause ventricular fibrillation (table 1). In the three enumerated experiments the change, though slight, was in the direction of a rise in the threshold. Excluding the first experiment, the average threshold for the unquinidinized animal in the entire group was 0.20 amperes; for the quinidinized animal, 0.60 amperes. The average for those animals in which ethyl carbamate anesthesia was used (excepting number 1) was 0.23 amperes in the unquinidinized condition and 0.67 amperes in the quinidinized condition. In the last five experiments of the group, in which the cats were pithed, the average before quinidine administration was 0.11 amperes and after quinidine administration 0.42 amperes. It is important to note that the threshold apparently bears no relation to the body weight of the cat. The average results of the entire group are probably less significant than the comparative results in a single experiment, because the quinidine threshold of one animal may be a lower value than the normal threshold of another. For example,

TABLE 2.—*Effect of the Excretion of Quinidine in Causing a Fall in the Threshold for Ventricular Fibrillation*

Exper. No.	Date	Weight, Kg.	Anesthesia	Threshold After Quinidine	Threshold After Excretion of Quinidine
19	8/11/30	1.3	Ethyl carbamate	0.50 amperes at 11:52	0.11 amperes at 1:34
20	8/12/30	3.6	Ethyl carbamate	0.40 amperes at 11:32	<0.10 amperes at 12:26
21	8/13/30	1.9	Ethyl carbamate	0.40-0.50 amperes at 11:45	0.25 amperes at 12:57 0.20 amperes at 1:54 0.25 amperes at 2:57
22	8/14/30	2.7	Ethyl carbamate	0.20 amperes at 11:11	0.15 amperes at 12:00
23	6/18/31	2.5	Decerebrate	1.00 amperes at 12:48	1.00 amperes at 2:30
24	6/29/31	3.1	Decerebrate	0.30 amperes at 11:53	0.05 amperes at 1:02
25	6/30/31	2.6	Decerebrate	>1.50 amperes at 12:06	1.50 amperes at 1:55
	Average			0.62 amperes	0.44 amperes

cats 4, 6 and 10 had normal thresholds of 0.35 amperes while cats 5 and 13 each had quinidine thresholds of 0.30 amperes.

In order to rule out the possibility that the previous treatment and manipulation of the heart was the cause of the rise of threshold, it was decided to reverse the procedure used in the first set of experiments. The quinidine was given first and the threshold determined after allowing adequate time for absorption. An hour or more later the threshold was again determined, when a good part of the quinidine had presumably been excreted.

With the exception of experiment 23, all the experiments in this series (table 2) show a fall in the threshold after sufficient time has been allowed to elapse for the quinidine to be excreted in whole or in part. Here, again, too much significance must not be given average results. It is noteworthy, however, that the average threshold for the quinidinized animals in this series was 0.62 amperes, which is approximately the same as the average of the first series, i. e., 0.60 amperes. The average after the excretion of the quinidine was 0.44 amperes.

This shows that the change in the threshold in the experiments is due to the quinidine and not to extraneous factors such as fatigue and manipulation.

In a third series of experiments the methods of the two previous sets were combined (table 3). The threshold was determined thrice, before, shortly after and a longer time after the injection of quinidine. With the exception of cat 28, in which quinidine was without effect, the administration of the drug was followed by a rise in the threshold current for ventricular fibrillation and then, with the passage of more time, by a fall in the threshold toward the original level.

TABLE 3.—*Effect of Absorption and Excretion of Quinidine in Causing a Rise and Fall in the Threshold for Ventricular Fibrillation*

Exper. No.	Date	Weight, Kg.	Anesthesia	Threshold Before Quinidine	Threshold After Quinidine	Threshold After Excretion of Quinidine
26	10/21/30	1.3	Ethyl carbamate	0.20 amperes at 3:42	0.60 amperes at 4:33	0.40 amperes at 5:35 0.30 amperes at 6:29
27	10/23/30	2.2	Ethyl carbamate	0.10 amperes at 3:36	0.20 amperes at 4:17	0.10 amperes at 5:02 (questionable figure)
28	2/16/31	2.3	Decerebrate	0.10 amperes at 2:59	0.10 amperes at 3:41	0.10 amperes at 4:08
29	4/ 8/31	2.4	Decerebrate	0.10 amperes at 10:40	0.40 amperes at 11:28	0.10-0.15 amperes at 12:21
30	6/ 3/31	2.2	Decerebrate	0.05 amperes at 11:13	0.10 amperes at 11:44	0.05 amperes at 12:37
31	6/ 5/31	2.5	Decerebrate	0.05 amperes at 11:38	0.20-0.30 amperes at 12:23	0.10 amperes at 1:34
32	6/30/31	2.6	Decerebrate	0.10 amperes at 2:28	1.50 amperes at 3:19	0.20 amperes at 4:06
Average				0.10 amperes	0.45 amperes	0.14+ amperes

Control experiments (table 4) were performed showing that there is no natural change or only a very slight change in the threshold for ventricular fibrillation during a period of two hours if no quinidine is given. Furthermore, the injection of distilled water or of physiologic solution of sodium chloride did not alter the threshold (experiments 35 and 36).

The work was prompted by the clinical results obtained in the treatment of ventricular tachycardia with quinidine.[17] In this regard it was found that the drug not only breaks up the abnormal rhythm but may prevent its return when the condition has a tendency to recur in paroxysms. As fibrillation of the ventricles may well have a similar

17. Levine, S. A., and Fulton, M. N.: The Effect of Quinidine Sulphate on Ventricular Tachycardia: Clinical Observations, J. A. M. A. **92**:1162 (April 6) 1929.

relation to ventricular tachycardia as auricular fibrillation has to auricular flutter, it seemed logical that quinidine might actually inhibit the development of ventricular fibrillation. It is well known that although sudden, instantaneous and unexpected death can occur, especially in coronary thrombosis from rupture of the ventricle or from sudden complete heart block, there are other instances where these two factors are inadequate to explain the mechanism of death. It is because such otherwise unexplained deaths may well be due to ventricular fibrillation that the experiments reported in this study may have a practical application for proper quinidine therapy or for some hope in inhibiting these fatalities.

TABLE 4.—*The Constancy of the Threshold with Lime and with the Administration of Control Solutions*

Exper. No.	Date	Weight, Kg.	Anesthesia	Determinations				
				First, Amperes	Second, Amperes	Third, Amperes	Fourth, Amperes	Fifth, Amperes
3	7/22/30	2.3	Ethyl carbamate	0.25 at 11:36	0.25 at 11:51	0.25 at 12:04	0.25 at 12:14	
7	7/28/30	1.7	Ethyl carbamate	0.30 at 11:40	0.30 at 11:55	0.30 at 12:10	0.30 at 12:25	0.30 at 12:45
33	4/ 5/30	Small cat	Ethyl carbamate	0.47 at 4:07	0.48 at 4:21			
34	11/ 4/30	2.0	Ethyl carbamate	0.15 at 12:12	0.10 at 1:06			
35	11/ 5/30	1.7	Ethyl carbamate; distilled water injected at 11:55	0.10 at 12:16	0.10 at 1:05	0.10 at 1:58		
36	11/ 6/30	1.9	Ethyl carbamate; 3.8 cc. saline injected at 11:37	0.10 at 11:55	0.10 at 12:55	0.10 at 1:59		

CONCLUSIONS

In a series of thirty-six experiments performed on cats, it was found that quinidine sulphate definitely inhibited the facility with which ventricular fibrillation could be produced by faradic stimulation. It was also found that this effect could not be attributed to manipulation of the heart and the resulting fatigue of the muscle or to the time consumed in the experiments.

It is suggested that these results offer a rational background for proper quinidine therapy as a method of preventing sudden death in those conditions in which ventricular fibrillation is prone to occur.

NOTE.—While this paper was in the hands of the printer, two pertinent articles were called to my attention. In one, Davis and Sprague [18] reported the occurrence of syncopal attacks in a patient with rheumatic

18. Davis, D., and Sprague, H. B.: Ventricular Fibrillation: Its Relation to Heart Block, Am. Heart J. **4**:559, 1929.

heart disease and auricular fibrillation. Following treatment with quinidine in an attempt to restore normal rhythm, electrocardiograms showed ventricular abnormalities consistent with ventricular fibrillation. In this paper the possible action of quinidine in initiating ventricular fibrillation is discussed. In the other, Dock [19] reported a case of recurrent attacks of syncope occurring over a period of eighteen months, due presumably to ventricular fibrillation. Subsequent quinidine medication prevented these attacks.

19. Dock, W.: Transitory Ventricular Fibrillation as a Cause of Syncope and Its Prevention by Quinidine Sulphate, Am. Heart J. **4**:709, 1929.

INTRINSIC REGULATION OF GASTRIC ACIDITY

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The mechanisms concerned in the lowering of gastric acidity following digestion have been the subject of considerable research since Pavlov [1] first noticed the apparent diminution of further secretion of hydrochloric acid when there was an accumulation of that substance within the gastric cavity. Although he did no further work on the subject, he originated the idea of intrinsic control of gastric acidity.

Rosemann [2] noted that although the concentration of total chloride remained constant, or relatively so, in specimens of gastric content, the acid chloride measured as hydrochloric acid and the neutral chloride measured as sodium chloride varied in inverse proportion. He advanced the hypothesis that as the stimulus to gastric secretion became stronger, the amount of chloride ion secreted as hydrochloric acid became greater, and the amount secreted as neutral chloride became correspondingly less. When the stimulus was absent or less marked, the reverse occurred. Earlier investigators, notably Pfaundler,[3] had noticed the increase in neutral chloride late in the digestive cycle when the acid chloride was decreasing, and attributed this rise to neutralization of secreted acid chloride by mucin and alkaline pyloric juices.

Boldyreff,[4] in 1907, suggested that gastric acidity was self-regulated by regurgitation of alkaline duodenal juices. His classic experiments need not be considered here; they have been confirmed many times by

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1. Pavlov, I. P.: The Work of the Digestive Glands, ed. 2, London, Griffin and Company, 1910.

2. Rosemann, R.: Beiträge zur Physiologie der Verdauung. I. Die Eigenschaften und die Zusammensetzung des durch Scheinfütterung gewonnenen Hundemagensaftes, Arch. f. d. ges. Physiol. **118**:467 (July) 1907.

3. Pfaundler, quoted by MacLean, Hugh; and Griffiths, W. J.: The Automatic Regulation of Gastric Acidity, J. Physiol. **66**:356 (Dec.) 1928.

4. Boldyreff, W.: Der Uebertritt des natürlichen Gemisches aus Pankreassaft, Darmsaft und Galle in den Magen, Arch. f. d. ges. Physiol. **121**:13 (Dec.) 1907.

Cathcart,[5] Spencer and his associates,[6] Bolton and his associates[7] and others. Olch and Elman[8] recently confirmed the part played by regurgitation, diverting or returning pancreatic juice from or to the duodenum at will with an apparatus devised by Elman and McCaughan.[9] They found that neutralization of an acid test meal was rapid when secretions were present in the duodenum and absent or markedly decreased after the diversion of the secretions.

Carlson,[10] Hicks and Visher[11] and others assumed that, whereas regurgitation into the stomach is a physiologic phenomenon, it is not the important mechanism in the lowering of gastric acidity. Carlson, because of observations made over a period of seven years on a man with an opening made at gastrostomy, considered that the concentration of secreted hydrochloric acid varies. Other investigators, notably Foster and Lambert[12] and Lim,[13] confirmed this observation.

Rosemann's[2] hypothesis was emphasized by the investigations of Katsch and Kalk,[14] Steinitz[15] and others. Hansman, Day and Clifton[16] expressed the belief that it is impossible to account for the increase in the concentration of neutral chloride by assuming neutralization of

5. Cathcart, E. P.: Reflux from Intestine to Stomach, J. Physiol. **42**:433 (July) 1911.

6. Spencer, W. H.; Meyer, G. P.; Rehfuss, M. E., and Hawk, P. B.: Gastro-Intestinal Studies: XII. Direct Evidence of Duodenal Regurgitation and Its Influence upon the Chemistry and Function of the Normal Human Stomach, Am. J. Physiol. **39**:459 (Feb.) 1916.

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11. Hicks, C. J., Jr., and Visher, J. W.: Contributions to the Physiology of the Stomach: XXVII. The Mechanism of Regurgitation of Duodenal Contents into the Stomach, Am. J. Physiol. **39**:1 (Nov.) 1915.

12. Foster, N. B., and Lambert, A. V. S.: Some Factors in the Physiology and Pathology of Gastric Secretion, J. Exper. Med. **10**:820 (Nov.) 1908.

13. Lim, R. K. S.: On the Relationship Between the Gastric Acid Response and the Basal Secretion of the Stomach, Am. J. Physiol. **69**:318 (July) 1924.

14. Katsch, G., and Kalk, Heinz: Statik und Kinetik des Magenchemismus, Arch. f. Verdauungskr. **32**:201, 1923.

15. Steinitz, Hermann: Die Chlorabscheidung des menschlichen Magens in ihrer Beziehungen zu pathologischen Vorgangen, Arch. f. Verdauungskr. **42**:57, 1928.

16. Hansman, F. S.; Day, Emily M., and Clifton, R.: Gastric Chlorides, Their Origin and Significance, M. J. Australia **2**:6 (July 27) 1927.

secreted acid chloride by all the available base in mucin, alkaline pyloric secretions and duodenal juice. MacLean and Griffiths [17] were also staunch supporters of this intrinsic mechanism, because of several observations. The neutralization of acid test meals may be prompt without evidence of regurgitation, either in staining of gastric content by bile or in discovery of tryptic activity in gastric content; acidity varies in a Pavlov pouch as it does in the intact stomach, although regurgitation into a Pavlov pouch is not possible. MacLean and Griffiths maintained that an accumulation of hydrogen ions above a certain level inhibits the further secretion of acid.

Baird, Campbell and Hern [18] prevented regurgitation by continuous removal of duodenal content by a suction apparatus, and found test meals to be unaltered. McCann [19] used Mann and Williamson's [20] method of duodenal drainage for the same purpose and found regulation of the acidity of the gastric content unchanged. He attributed reduction in the acidity of the gastric content to the gradual reduction of a high rate of secretion to a low rate, and to the capacity of mucus to combine with acid to form a combined acid and a neutral chloride, the concentration of which rises at the end of digestion.

In a comprehensive review of the subject Babkin [21] stated his belief that the curve of acidity is the net result of several important regulatory factors. He considered variation of the concentration of secreted acid, and contributed evidence of its significance. He expressed the belief that regurgitation is important and that secretion of neutral chloride plays a part. To gastric mucin, saliva and foodstuffs he attributed minor rôles.

This work was started primarily to determine the buffering capacity of the gastric juice to acids instilled into gastric pouches. It was believed that if the acidity of solutions that had been instilled became reduced, under experimental conditions that precluded the possibility of its being neutralized by food, saliva or regurgitated material, one could assume the existence of intrinsic regulation of gastric acidity. By following the titratable acidity and the chloride content within the pouch, one might obtain information regarding the nature of this mechanism.

17. MacLean, Hugh, and Griffiths, W. J.: The Automatic Regulation of Gastric Acidity, J. Physiol. **66**:356 (Dec.) 1928.

18. Baird, M. McC.; Campbell, J. M. H., and Hern, J. R. B.: The Importance of Estimating Chlorides in Fractional Test Meal Samples, and Some Experiments with the Duodenal Tube, Guy's Hosp. Rep. **74**:23 (Jan.) 1924.

19. McCann, J. C.: Studies on Control of Acidity of Gastric Juice, Am. J. Physiol. **89**:483 (Aug.) 1929.

20. Mann, F. C., and Williamson, C. S.: The Experimental Production of Peptic Ulcer, Ann. Surg. **77**:409 (April) 1923.

21. Babkin, B. P.: Physiological Factors Determining Acidity of Gastric Juice and of Gastric Contents, Canad. M. A. J. **17**:36 (Jan.) 1927.

METHODS OF INVESTIGATION

Operative procedures were all carried out on dogs under ether anesthesia and with aseptic technic. Pyloric pouches were prepared by a modified Polya type of resection in two stages (figs. 1 and 2). Little difficulty was encountered; the pouches readily retained solutions instilled through a Pezzer mushroom retention catheter.

With ordinary Pavlov pouches of the fundus, it was almost impossible to prevent excoriation of the skin, and this difficulty was best obviated by constructing pouches by a modification of Mann and Bollman's [22] method of preparing an intestinal fistula.[23] Essentially a loop of resected ileum was employed as a fistulous tract to drain the fundic pouch. Anastomosis of the distal end of the loop to the stomach produces a valvelike effect, which prevents leakage and makes excoriation negligible. The possible objection that the relatively alkaline secretion of the loop might interfere with the experimental procedure by being regurgitated into the pouch was removed by inserting a mushroom retention catheter well within the pouch and exerting traction on it until the mushroom impinged strongly against the somewhat contracted line of the gastro-ileac anastomosis. Results in these animals were in every way comparable to those in which the pouches were attached directly to the abdominal wall.

The animals were trained until they became content to lie on a table for hours without restraint, which to a large degree eliminated any possible psychic interference with secretion. Two series of experiments were done, each on animals with pyloric and fundic pouches, both after fasting and after test meals of 100 Gm. of meat and 250 cc. of water. In the first series, from 10 to 15 cc. of hydrochloric acid of known reaction was instilled into a pouch, and about 1 cc. was withdrawn at intervals of half an hour. The reaction of these specimens was determined by the quinhydrone electrode as described by Mann and Bollman. These determinations were done to decide whether readings of reaction were a true index of acidity of the content.

In the second series of experiments fractional specimens of the contents of the pouch were not analyzed. Determinations of changes in reaction and in free, combined and total acidity were made. Hydrochloric acid of known strength (approximately twentieth-normal) was instilled into a pouch and allowed to remain therein for from one to three and a half hours in different experiments, and then was removed. Free and total acidity were determined by titration against tenth-normal sodium hydroxide, and the content of chloride by precipitation with a known amount of silver nitrate and by titration of the excess silver against an equivalent solution of ammonium thiocyanate. Differences in volume were noted as evidence of secretion or possible absorption. These experiments were repeated with sulphuric acid.

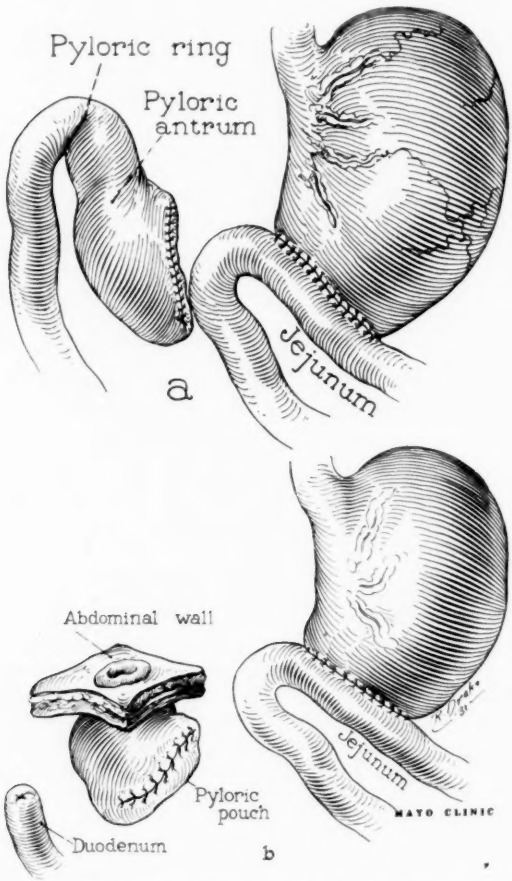
RESULTS

The variations in reaction throughout the first series were not marked. In the pyloric pouches there was a rise in acidity averaging

22. Mann, F. C., and Bollman, J. L.: Reaction of Content of Gastro-Intestinal Tract, J. A. M. A. **95**:1722 (Dec. 6) 1930; A Method for Making a Satisfactory Fistula at Any Level of the Gastro-Intestinal Tract, Ann. Surg. **85**:794 (March) 1931.

23. Goldberg, S. L., and Mann, F. C.: A Satisfactory Method of Preparing Fundus Gastric Pouches, Ann. Surg. **94**:953 (Nov.) 1931.

approximately 0.6 of a p_H unit; in the group of animals with fundic pouches the increase was somewhat less (table 1). The second series of experiments was more fruitful. In an experiment in which 10 cc. of hydrochloric acid of known strength was injected into a pyloric



The first stage in making a pyloric pouch (after Priestley and Mann) is indicated by *a*, the completed pyloric pouch by *b*.

pouch and allowed to remain for one hour, the chloride content invariably rose, at times 100 per cent or more. The total acidity varied but little; this could be explained adequately by dilution, which at best was not marked. Within an hour, however, about 20 per cent of the free acidity became combined, reaching 40 per cent in some experiments

(table 2). The same experiments on dogs with fundic pouches gave comparable results (table 3), showing the regulatory mechanism common to both portions of the stomach. In practically every experiment the volume of the content increased, thus showing that secretion into

TABLE 1.—*Determinations of the p_H of Samples Removed from Gastric Pouches at Intervals After Instillations of Acid*

	Pyloric Pouch		Fundic Pouch	
	Fasting	After Test Meal	Fasting	After Test Meal
Initial p_H of content of pouch	6.27	6.36	4.73	4.52
p_H of acid instilled*	1.41	1.41	1.64	1.64
p_H of content of pouch				
Thirty minutes later	1.54	1.59	1.71	1.69
One hour later	1.67	1.75	1.75	1.73
One and five-tenths hours later	1.75	1.92	1.81	1.75
Two hours later†	2.12	2.12	1.89	1.83

* The amount of acid instilled was 10, 10, 15 and 15 cc., respectively.
† The amount of acid recovered was 4, 4, 10 and 13 cc., respectively.

TABLE 2.—*Changes in 10 Cc. of Hydrochloric Acid Instilled into a Pyloric Pouch of a Dog and Removed After One Hour*

	Volume, Cc.	Acidity*			Chlorides, Mg. for Each 100 Cc.	p_H
		Free	Combined	Total		
Fasting						
Acid instilled	10.0	28	0	28	220	1.94
Content removed	11.0	20	5	25	360	2.00
After Feeding						
Acid instilled	10.0	28	0	28	220	1.94
Content removed	11.5	20	5	25	330	2.01

* In all tables acidity is expressed in terms of cubic centimeters of tenth-normal sodium hydroxide required to produce end-point in 100 cc. of content.

TABLE 3.—*Changes in 10 Cc. of Hydrochloric Acid Instilled into a Fundic Pouch of a Dog and Removed After One Hour*

	Volume, Cc.	Acidity			Chlorides, Mg. for Each 100 Cc.	p_H
		Free	Combined	Total		
Fasting						
Acid instilled	10	28	0	28	220	1.94
Content removed	11	20	4	24	450	2.06
After Feeding						
Acid instilled	10	28	0	28	220	1.94
Content removed	12	18	6	24	400	2.02

the pouch had taken place. Feeding had little apparent effect on the results.

All the preceding experiments in the second series were repeated, an approximately twentieth-normal solution of sulphuric acid being used. There was an invariable rise in the chloride content from the initial zero, and changes in acidity were similar to those obtained when hydrochloric acid was used, which is evidence that the presence of a

high enough concentration of hydrogen ions inhibits the secretion of acid by the stomach, regardless of what negative radical it may be linked with (tables 4 and 5).

To determine if possible the extent to which the drop in free acidity would take place, acid was left in pouches for varying periods up to three and a half hours. In an experiment performed after feeding an

TABLE 4.—*Changes in 10 Cc. of Sulphuric Acid Instilled into a Pyloric Pouch of a Dog and Removed After One Hour*

	Volume, Cc.	Acidity			Chlorides, Mg. for Each 100 Cc.	*p*H
		Free	Combined	Total		
Fasting						
Acid instilled	10	50	0	50	...	1.97
Content removed	11	40	5	45	220	2.12
After Feeding						
Acid instilled	10	50	0	50	...	1.97
Content removed	12	36	8	44	220	2.21

TABLE 5.—*Changes in 10 Cc. of Sulphuric Acid Instilled into a Fundic Pouch of a Dog and Removed After One Hour*

	Volume, Cc.	Acidity			Chlorides, Mg. for Each 100 Cc.	*p*H
		Free	Combined	Total		
Fasting						
Acid instilled	10	50	0	50	...	2.02
Content removed	11	32	12	44	180	2.11
After Feeding						
Acid instilled	10	50	0	50	...	2.02
Content removed	13	26	12	38	220	2.12

TABLE 6.—*Changes in 10 Cc. of Hydrochloric Acid Instilled into a Fundic Pouch of a Dog After Feeding and Removed After Three and One-Half Hours* *

	Volume, Cc.	Acidity			Chlorides, Mg. for Each 100 Cc.	*p*H
		Free	Combined	Total		
Acid instilled	10	28	0	28	220	2.18
Content removed	12	0	24	24	473	5.16

* Complete combination of free acid, drop in total acidity in proportion to dilution, and increase in content of chloride.

animal with a fundic pouch, in which hydrochloric acid was used, the free acid became totally combined in three and a half hours. The total acidity dropped somewhat, but only in proportion to the degree of dilution, and the chloride content rose (table 6).

COMMENT

Changes in the reaction of acids instilled into pouches, although present, were not sufficient to permit conclusions to be drawn. It was

somewhat surprising not to find more definite evidence of buffer activity on the part of the gastric juice, particularly of the pyloric secretions, such as one might expect in the light of previous work. It was reasoned that inasmuch as there was some change in reaction, and more in pyloric than in fundic pouches, possibly these determinations alone were not suitable indexes of the chemical nature of the content of the pouch. Subsequent results seemed to strengthen this view.

Secretion of a neutral chloride seems to be the only explanation of the rise in chloride content. Regurgitated duodenal juices and swallowed saliva are eliminated as possible sources of chlorides by the method of procedure. Another possible cause is neutralization after secretion by the gastric mucosa as hydrochloric acid. If this were the explanation, the total acidity should be increased, a condition that was not found in any experiment. As far as present knowledge of gastric physiology is concerned, it must be assumed that secretion of a neutral chloride explains these results.

Such secretion probably has little effect on the acidity of the gastric content. There may be some reduction by dilution, but this at best is not remarkable, and it is known that the buffer capacity of the sodium chloride is negligible. It is only a corollary of the more important mechanism, that of decrease in the concentration of the hydrochloric acid secreted by the mucosa of the stomach when the gastric content becomes highly acid. The nature of the acidity is unimportant, inasmuch as sulphuric acid had the same inhibitory effect. The old idea of Pavlov and of other early investigators that hydrochloric acid is secreted at a constant concentration has been almost discarded. Foster and Lambert, Carlson, Lim, Babkin and others have since shown that variations can and do occur. Hollander and Cowgill [24] recently concluded that the concentration of the secretion of hydrochloric acid is constant. They found a constant upper level of acidity which they believed was determined by the osmotic pressure of the blood serum. The second series of experiments strengthens the view that variations occur, as in the actively secreting pouch the secretion of hydrochloric acid was either considerably or entirely suppressed, and the secretion of a neutral chloride took place. Although a rise in total acidity was not found, this does not definitely establish that the low level of secretion of hydrochloric acid is zero. It was Lim's belief that the low level is 0.29 per cent. Also it is difficult to determine whether any definite ratio exists between the neutral and the acid chlorides secreted. If the total secretion of chloride is assumed to be constant, there may be a

24. Hollander, Franklin, and Cowgill, G. R.: Studies in Gastric Secretion: I. Gastric Juice of Constant Acidity, J. Biol. Chem. **91**:152 (April) 1931.

definite ratio, but although many of the recent workers believe this to be true, the fact has not as yet been established.

The drop in free acidity with the concurrent rise in combined acidity must be explained, in the light of present knowledge, as the result of combination of acid with mucin, as was Foster's [25] contention. Recent work by Fogelson [26] showed that 1 Gm. of mucin will combine with from 12 to 15 cc. of tenth-normal hydrochloric acid, and mucin was the only known substance present in these pouches that could combine with free acid in the experimental procedure used. The part it plays becomes more important when the rate of secretion is low, as the constant basal secretion combines with a greater proportion of the total secretion at such a time. The so-called "alkaline pyloric juice" is practically entirely mucus, as shown by Ivy and Oyama [27] and by Lim and Dott.[28]

The extragastric factors that play a part in this regulation must be considered. Few, if any, physiologic mechanisms are influenced or regulated by only one factor, and the regulation of gastric acidity is undoubtedly no exception to the general rule. Indeed, it is fortunate that several factors are involved. That regurgitation of alkaline duodenal juices does occur cannot be doubted, and that some neutralization takes place following this is axiomatic. It should be considered an associated phenomenon and not the only or most important factor, as its advocates would make it, for it is known that regulation is adequate without it. It is impossible even to guess at the relative significance of the extragastric and intragastric mechanisms at any particular time. When there is a large gush of alkaline fluids through the pylorus, this may become momentarily more significant; when regurgitation does not take place, the intragastric mechanisms are more significant.

Swallowed saliva may reduce acidity, although it may play only a minor part. It is estimated [29] that 1,500 cc. of saliva is swallowed daily. By virtue of its volume alone, its mucin content and its buffer salts,[30] it is conceivable that the swallowed saliva might be a minor

25. Foster, N. B.: The Chemical Affinity of Mucus for Hydrochloric Acid, Am. J. M. Sc. **133**:303 (Feb.) 1907.

26. Fogelson, S. J.: The Treatment of Peptic Ulcer with Gastric Mucin: Preliminary Report, J. A. M. A. **96**:673 (Feb. 28) 1931.

27. Ivy, A. C., and Oyama, Yutaka: Studies on the Secretion of the Pars Pylorica Gastri, Am. J. Physiol. **57**:51 (Aug.) 1921.

28. Lim, R. K. S., and Dott, N. M.: Observations on the Isolated Pyloric Segment and Its Secretion, Quart. J. Exper. Physiol. **13**:159, 1922.

29. Bidder and Schmidt, quoted by Mathews, A. P.: Physiological Chemistry, ed. 3, New York, William Wood & Company, 1923.

30. Marshall, J. A.: Etiology of Dental Caries, Physiol. Rev. **4**:564 (Oct.) 1924.

factor. McClendon [31] has expressed the belief that saliva may completely neutralize acidity when the stomach is almost empty.

Ingested food stuffs reduce acidity by absorption, by actual neutralization if they are alkaline and by dilution. If the buffer capacity of a particular food is sufficient, this may be a factor, at best relatively unimportant.

SUMMARY

Acid of known reaction, titratable acidity and chloride content was instilled into isolated pyloric and fundic pouches of fasting and actively secreting dogs, and after varying intervals these factors were redetermined and changes noticed.

Determinations of reaction by themselves are not an adequate index of chemical changes in the gastric content.

The buffering ability of the secretions of the pyloric segment is somewhat better than that of the fundus.

Evidence is produced to show that intragastric regulation of acidity is probably brought about by three factors: (1) reduction of the concentration of secreted hydrochloric acid when intragastric acidity has reached a certain level, and, as its corollary (2) secretion of neutral chloride by the stomach, which plays a rather minor part by dilution, and (3) a combination of gastric mucin or an unknown substance with free acid.

31. McClendon, J. F.: Acidity Curves in the Stomachs and Duodenums of Infants, Plotted with the Aid of Improved Methods of Measuring Hydrogen Ion Concentration, Am. J. Physiol. **38**:191 (Aug.) 1915.

CHEMISTRY AND METABOLISM IN EXPERIMENTAL YELLOW FEVER IN MACACUS RHESUS MONKEYS

V. ACID-BASE AND ELECTROLYTE EQUILIBRIUM

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AND
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It has already been pointed out that in the last stages of fatal yellow fever in Macacus rhesus the urinary excretion of organic acid and phosphates is considerably increased.[1] In the present paper are reported the results of more extensive studies of the acid-base and electrolyte equilibrium in this disease.

METHODS

The general experimental procedures outlined in the first paper of this series [2] have been carefully followed.

The electrolytes of the serum were determined by procedures essentially the same as those described by Peters, Wakeman, Eisenman and Lee.[3] Blood was withdrawn and serum separated, with anaerobic precautions, by the technic of Austin and his co-workers.[4]

Blood was collected without stasis from the heart, the femoral artery or the veins of the upper extremities. In certain of the normal animals attempts were made to withdraw venous blood without anesthesia. However, it soon became evident from the low values for carbon dioxide that this method could not be employed. The reduction of

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1. Wakeman, A. M., and Morrell, C. A.: Chemistry and Metabolism in Experimental Yellow Fever in Macacus Rhesus Monkeys: II. Nitrogen Metabolism, Arch. Int. Med. **46**:382 (Sept.) 1930.

2. Wakeman, A. M., and Morrell, C. A.: Chemistry and Metabolism in Experimental Yellow Fever in Macacus Rhesus Monkeys: I. Concentration of Nonprotein Nitrogenous Constituents in Blood, Arch. Int. Med. **46**:290 (Aug.) 1930.

3. Peters, J. P.; Wakeman, A. M.; Eisenman, A. J., and Lee, C.: J. Clin. Investigation **6**:517, 1929.

4. Austin, J. H.; Cullen, G. E.; Hastings, A. B.; McLean, F. C.; Peters, J. P., and Van Slyke, D. D.: J. Biol. Chem. **54**: 121, 1922.

carbon dioxide was apparently due to the exercise of struggling. In one experiment, samples of blood taken at intervals showed a progressive fall in carbon dioxide as struggling continued. It therefore proved necessary to use amytal anesthesia. Bolliger and Maddox [5] and Chambers, Deuel and Milhorat [6] have shown that amytal does not alter the carbon dioxide absorption curve of the blood. In one experiment, blood taken under local anesthesia from the vein of a monkey that remained quiet during the procedure, was found to contain 53.8 per cent by volume of carbon dioxide. Blood withdrawn one hundred and sixty minutes later when the animal was thoroughly anesthetized with amytal contained 54.3 per cent by volume. It was therefore concluded that the effect of amytal on the carbon dioxide content of the blood was not great enough to influence experimental data significantly. In most experiments on monkeys with yellow fever, anesthesia was employed. In a few instances moribund animals, too stuporous and prostrate to offer any resistance to the procedure, were bled without the production of anesthesia or when a local anesthetic was used. It is believed that values for carbon dioxide secured under these conditions were comparable to those secured from active animals under amytal. Serum proteins appeared to be inappreciably affected by struggling and anesthesia.

Carbon dioxide of serum was determined by the method of Van Slyke and Neill;[7] chloride, by the procedure of Van Slyke;[8] calcium, by that of Kramer and Tisdall,[9] and inorganic phosphorus, by that of Fiske and Subbarow.[10] Total base was determined by a modification of Stadie and Ross' adaptation of Fiske's urine method.[11] For the determination of proteins 1 cc. of serum was diluted to 25 cc. with physiologic solution of sodium chloride, and 10 cc. aliquots were subjected to a macro-Kjeldahl procedure. Albumin and globulin fractions were separated by the method of Howe [12] and analyzed in the same manner.

The base-combining power of the various acid fractions was estimated by means of the factors employed by Peters, Wakeman, Eisenman and Lee.[3]

5. Bolliger, A., and Maddox, K.: M. J. Australia **1**:510, 1930.
6. Chamber, W. H.: Deuel, H. J., Jr., and Milhorat, A. T.: J. Biol. Chem. **75**:422, 1927.
7. Van Slyke, D. D., and Neill, J. M.: J. Biol. Chem. **59**:523, 1924.
8. Van Slyke, D. D.: J. Biol. Chem. **58**:523, 1923.
9. Kramer, B., and Tisdall, F. F.: J. Biol. Chem. **47**:475, 1921.
10. Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. **66**:375, 1925.
11. Stadie, W. C., and Ross, E. C.: J. Biol. Chem. **65**:735, 1925.
12. Howe, J. P.: J. Biol. Chem. **49**:93, 1921.

EXPERIMENTAL RESULTS

The only important electrolyte disturbances that seemed to be distinctly referable to yellow fever involved protein, bicarbonate, phosphate, the undetermined acid fraction and calcium. Total base showed a larger range of variation among animals with yellow fever than among normal animals, but there was no especial tendency to either reduction or increase of the bases. For the most part chloride remained unaltered during yellow fever. In two instances considerable reductions were encountered, 91.7 milliequivalents and 89.5 milliequivalents, respectively. In both of these instances bicarbonate was also greatly reduced. There is no evidence in the experiments of excessive loss of chloride by vomiting or by other extrarenal modes of excretion. It seemed probable, therefore, that the chloride reductions in these cases were comparable to those described by Peters and his associates [13] in diabetic acidosis, chloride apparently yielding its base to aid bicarbonate in the neutralization of foreign acids.

In chart 1 the proteins of normal monkeys and of monkeys with yellow fever are compared. The abscissas in this chart represent the percentage of protein in serum. The ordinates in the two lower sections of the chart indicate the number of observations. The spread of serum protein in normal monkeys seems very great. The two determinations below 5 per cent, 4.46 and 4.39 per cent, respectively, were obtained on the same day from two small monkeys used for experiments with glycogen which have been described in an earlier paper of this series. The great discrepancy between these two observations and the others, coupled with the fact that both were determined on a single day, suggests the introduction of some analytic error. Of the four determinations in which the proteins were below 5.7 per cent, one was secured from a monkey that was recognized to be distinctly sickly and two came from random studies of monkeys taken from the normal stock cage. The term "normal" as applied to those monkeys is entirely a relative one; all that can be said with certainty is that they were not suffering from yellow fever or any obvious serious illness. Of the twenty-eight observations made, twenty-one lay between 5.7 and 7.4 per cent. It is probable that this more nearly defines the limits of variation in truly normal monkeys, although in animals selected as were those used for studies on yellow fever these limits probably must be extended down to 5 per cent.

The middle portion of the chart (II) shows the serum proteins in monkeys with yellow fever. The numerals in the squares indicate the

13. Peters, J. P.; Bulger, H. A.; Eisenman, A. J., and Lee, C.: J. Clin. Investigation **2**:167, 1925.

day of the disease estimated from the time when the temperature first became significantly elevated. In four instances, indicated by interrogation marks, the day of the disease was unknown because the monkeys were taken at random from stock yellow fever animals. It is at once apparent from the chart that the serum proteins are greatly reduced during the course of yellow fever, and that the reduction has a general

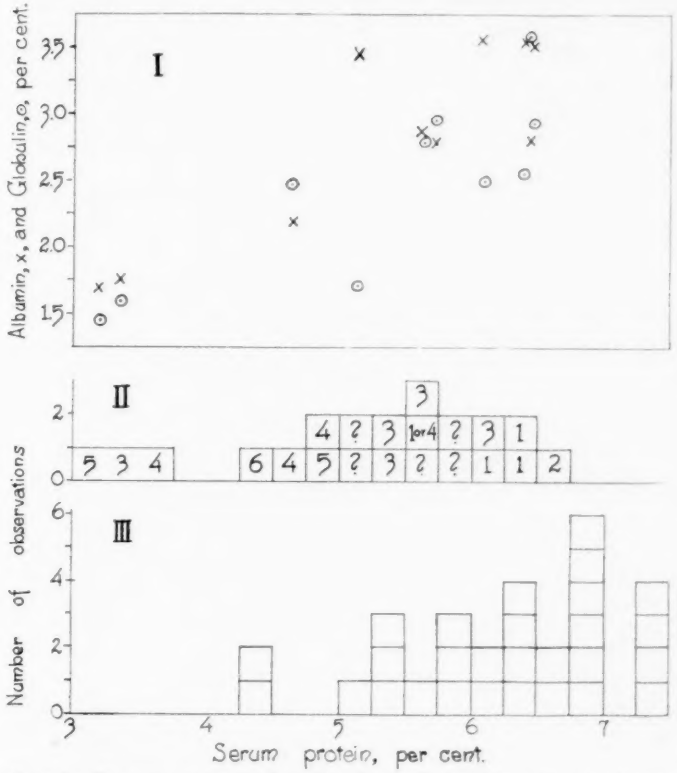

Chart 1.—The serum proteins in yellow fever. The abscissas represent percentage of proteins. In *I*, the ordinates represent per cent of albumin (*x*) and globulin (⊙) in monkeys with yellow fever. In *II* and *III* the ordinates represent number of observations in monkeys with yellow fever and in normal monkeys, respectively. The numerals in the squares in *II* indicate the duration of the disease in days, calculated from the onset of fever.

tendency to increase with the duration of the disease. In general, the low figures, indicating short febrile periods, lie to the right of the chart, the high figures, longer febrile periods, to the left. There are some exceptions to this rule. This may be due to variations in the severity

of the disease, which cannot be evaluated, and the fact that the onset of fever is not a perfect criterion of the onset of the disease.

In the top section of chart 1, the abscissa again represents the percentage of total proteins. The ordinate, on the other hand, represents the percentage of albumin and globulin found in those instances in which the proteins were fractionated. Observations on two normal monkeys indicate that the proportions of albumin and globulin in the serum of macacus are quite similar to those reported in human serum. It is apparent that as the proteins fall the concentration of albumin steadily diminishes. There is apparently a parallel fall in globulin. However, it becomes evident on further analysis that the globulin fraction during the first days of the disease, when the proteins are still high, has a tendency to lie above the normal level, while albumin often shows signs of diminution. One can conclude that in the early stages

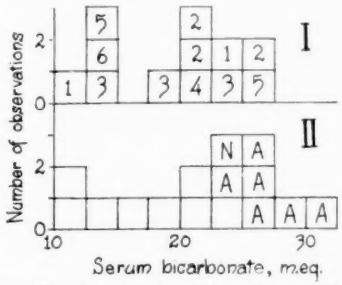

Chart 2.—The serum bicarbonate in yellow fever. The abscissas represent milliequivalents of serum bicarbonate. Ordinates represent the number of observations in monkeys with yellow fever (*I*) and normal monkeys (*II*). The numerals in the squares in *I* indicate the duration of the disease in days, calculated from the onset of fever. The letters in the squares in *II* indicate that amytal (*A*) or local anesthesia (*N*) was employed.

of the disease there is an initital rise of globulin which passes off as the disease progresses, while albumin is steadily depleted from the onset to the termination.

In chart 2, the serum bicarbonate of normal monkeys (II) is compared with that of animals with yellow fever (I). In the normal series the blank squares represent animals bled without anesthetic; squares marked *A*, animals anesthetized with amytal; and the one square marked *N*, the observation noted before in which local anesthesia was employed. For reasons already discussed, it is believed that only the data secured under anesthesia can be considered as showing a normal content and can be used for comparison with the figures for yellow fever. The normal range covered by these figures coincides with the normal limits of variation found in human beings.

There is an obvious tendency for bicarbonate to decrease in the course of yellow fever. However, this fall is not quite so consistent nor so closely related to the duration and severity of the disease as is the reduction in protein.

Inorganic phosphate of serum was frequently, but not invariably, increased in the terminal stages of yellow fever. In eight determinations on normal animals, values of serum phosphorus varied from 2.5 to 7 mg. per hundred cubic centimeters (from 1.5 to 4 milliequivalents). In nine determinations on monkeys with yellow fever, four were above this limit, varying from 8.6 to 15.7 mg. per hundred cubic centimeters (from 5 to 9.1 milliequivalents). In most instances high values for phosphate were associated with striking reductions in bicarbonate. This

TABLE 1.—*Total Serum Electrolyte Balance in Monkeys with Yellow Fever*

No.	Day of Fever	Protein, Milli-equiva-lents	Bicar-bonate, Milli-equiva-lents	Chlorides, Milli-equiva-lents	Inorganic Phos-phate, Milli-equiva-lents	Base, Milli-equiva-lents	Deter-mined Acids, Milli-equiva-lents	Unde-termined Acids, Milli-equiva-lents	Comment
1	3	8.1	14.3	91.7	5.0	152.0	119.1	32.9	Just after death
2	6	10.7	14.2	108.2	2.9	164.0	136.0	28.0	Moribund
3	2	15.8	22.4	113.0	...*	177.0	(153.5)	(23.5)	First day of fever
4	3	12.9	18.8	112.0	...*	158.6	(146.0)	(12.6)	Still active
5	1	15.6	11.4	124.5	9.1	180.5	160.6	19.9	Prostrated
6	4	8.7	21.0	110.0	1.9	163.5	141.6	21.9	Prostrated
7	3	13.1	24.7	109.6	3.4	159.5	150.8	8.7	Still lively
8	1	15.5	24.9	109.6	5.3	163.5	145.3	18.2	Still lively
9	5	7.8	14.5	89.5	5.7	149.0	117.5	31.5	Prostrated

* In these cases inorganic phosphate was not determined. For the calculation of total and undetermined acids it is assumed that the concentration of phosphate in the serum was 2.5 milliequivalents.

is illustrated in table 1. It is, however, also evident from this table that the acidosis is not caused chiefly by accumulation of phosphate.

In presumably normal animals, calcium was extremely variable, ranging from 7.6 to 10.6 mg. per hundred cubic centimeters. The majority of observations lay within the limits usually accepted as normal for human beings, from 9.6 to 10.6 mg. per hundred cubic centimeters. In three instances of twelve, in yellow fever, calcium values were lower than any observed in normal monkeys. The observations were made in such a random manner that it is impossible to correlate these reductions with disturbances in other factors or to establish any certain connection between them and the disease. The great majority of figures for serum calcium in yellow fever distribute themselves in much the same manner as do the figures obtained from normal subjects.

EXCRETION OF ACID METABOLITES

The excretion of chlorides, inorganic phosphorus and organic acids has been measured during control, febrile and terminal periods in

TABLE 2.—*Chloride Phosphorus and Organic Acid Excretion*

Date	Diet, Mg.	Urine: Chlorides in Urine, Mg.	Balance, Mg.	Urine: Organic Acid, N/10 Cc.	Urine: Phosphorus, Mg.	Serum Chloride, Mg. NaCl × 100 Cc. Serum	Urine Volume, Cc.
M. Rhesus M3							
September 1	520	370	150	28	12	612	..
2	520	720	—200	50	5	...	..
3	520	710	—190	58	6	...	..
4	520	490	30	49	13	...	..
5	520	700	—180	60	9	...	..
		2,990	—390	245	45		
Average	520	600	— 78	49	9		
September 6	520	276	244	55	14	631	..
7	520	223	297	62	28	...	..
8	520	308	212	50	15	...	..
9	520	430	90	54	11	...	..
		1,237	843	221	68		
Average	520	310	210	55	17		
September 11	520	134	386	95	25	648	..
12	520	321	199	..	81	...	..
13	520	170	350	52	12	...	..
		625	935	147	118		
Average	520	208	312	73	39		
September 14	520	83	437	49	21	...	..
15	520	...	...	..	..	...	..
16	520	350	170	37	6	650	..
		433	607	86	27		
Average	520	216	303	43	13		
M. Rhesus M5							
September 18	450	364	86	..	..	609	41
19	450	398	52	..	..	...	51
20	450	413	37	58	2	...	54
21	450	474	—24	51	2	...	45
		1,649	151	109			
Average	450	412	38	54	2		
September 23	450	252	198	..	..	628	26
24	450	527	—77	..	2	...	49
25*	450	454	— 4	62	3	...	66
26*	450	416	34	59	13	...	49
27	450	405	45	..	..	...	..
		2,054		121			
Average	450	411	19	60	6		
September 28*	450	276	174	..	..	644	90
29	225	42	183	111	64	...	53
	675	318	357				
Average	337	159	178	111	64		
M. Rhesus M10							
October 27	473	1,100	—627	72	26	...	91
28	473	666	—193	61	28	...	77
29	473	500	— 28	50	..	...	81
30	473	280	193	51	..	...	..
November 2	473	148	325	46	35	...	41
3	450	241	209	47	..	...	43
		2,935	—121	327	89		333
Average	469	492	— 21	54	30		66
November 4*	322	146	182	48	53	...	57
5*†	236	95	141	45	..	...	58
	558	241	323				115
Average	279	120	161	47	49		57

* Animal showed fever.
† Died at 11:15 p. m.

monkeys infected with yellow fever. Results of three such experiments are presented in table 2.

Macacus rhesus M3 was a large healthy male fed on diet no. 1.[2] It was inoculated on September 1 and again on September 10. The animal had a fever on September 5 and 11, but recovered and was subsequently immune to yellow fever.

Macacus rhesus M5 was a large, healthy female fed on diet no. 2. Inoculated on September 22, it showed fever on September 25, 26 and 28, and died on September 30.

Macacus rhesus M10 was a small, unhealthy female fed on diet no. 3. It was inoculated on November 2 and died on November 5 after one day of fever.

In the three examples presented for consideration two animals died of yellow fever and one recovered from the disease.

The excretion of chloride in the urine of M3 during the first control period was greater by 80 mg. per day than the intake of chloride; therefore the balance was negative. During the three remaining periods, however, there was a positive balance with no significant variation between normal and febrile periods. Organic acid and phosphorus were excreted in much larger quantities in the febrile than in the normal periods.

Essentially the same results were obtained in the two fatal cases, namely, an increase in the output of organic acid and phosphorus during the disease, with relatively no variation in the chloride in the urine.

COMMENT

These experiments seem to have established the fact that in yellow fever the serum proteins tend to fall steadily, chiefly at the expense of albumin, after an initial rise of globulin. Determinations of fibrinogen, which will be reported later, reveal striking increases in this protein fraction. It is not unlikely that the early increases in globulin and fibrinogen are merely responses to an inflammatory process. Similar increases have been reported in most inflammatory or infectious processes.[14] The pathogenesis of the deficit in albumin is less clear. Urinalyses on a large number of monkeys revealed relatively slight albuminuria. Therefore, it seems unlikely that loss of albumin through the kidneys can play an important rôle. Although it has been shown that nitrogen destruction is greatly increased in the course of the disease,[1] the duration of yellow fever is so brief that malnutrition of a

14. Bruckman, F. S.; D'Esopo, L. M., and Peters, J. P.: J. Clin. Investigation **8**:577, 1930. Rowe, A. H.: The Albumin and Globulin Content of Human Blood Serum in Health, Syphilis, Pneumonia and Certain Other Infections, Arch. Int. Med. **18**:455 (Oct.) 1916.

sufficient degree to affect serum albumin greatly is somewhat unlikely. In pneumonia in human beings, in spite of extreme toxic destruction of protein, serum albumin is usually only slightly reduced.[15]

From table 1 it is possible to evaluate the various factors that may be responsible for the deficit of bicarbonate in yellow fever. The lack of correlation between bicarbonate and either base of chloride has already been mentioned. Although in many instances phosphate plays a significant rôle in the production of the acidosis, it is not usually the most important factor and may remain normal even when bicarbonate

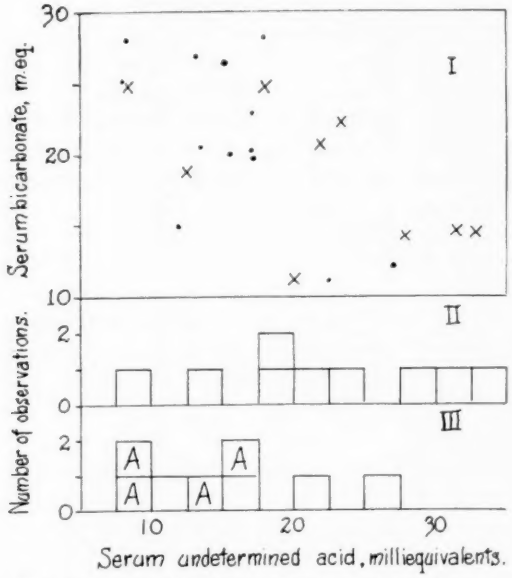

Chart 3.—The undetermined acid of serum in yellow fever. The abscissas represent milliequivalents of undetermined acid in serum. In *I* the ordinates represent milliequivalents of bicarbonate in normal monkeys (indicated by dots) and in monkeys with yellow fever (*x*), respectively. In *II* and *III* ordinates represent the number of observations in monkeys with yellow fever (*II*) and in normal monkeys (*III*), respectively. The letters *A* in the squares in *III* indicate that amytal anesthesia was employed.

is greatly reduced. The one element of the acid-base balance which is consistently related to the deficit in bicarbonate is the undetermined acid fraction. In chart 3, which is composed like chart 2, II and III represent the concentrations of undetermined acid in monkeys with

15. Sunderman, F. W.; Austin, J. H., and Camac, J. G.: J. Clin. Investigation **3**:37, 1926.

yellow fever and in normal monkeys, respectively. Among the normal animals only those marked *A*, i. e. the anesthetized animals, can properly be compared with the animals having yellow fever. In the latter there is a tendency for the undetermined acid fraction to increase as the disease progresses. In section I of the chart the increase of undetermined acids is seen to be inversely related to the bicarbonate deficiency. In this chart the dots represent comparisons made in normal monkeys; the crosses, those made in animals with yellow fever. High values for undetermined acids and low values for bicarbonate among the normal animals were found in those that were unanesthetized. The undetermined acid is presumably lactic acid, a product of exercise. The general trend of the relation between undetermined acid and bicarbonate in animals with yellow fever is much like the similar relation in normal animals.

From the fact, already demonstrated,[1] that urinary organic acid increases in yellow fever, it is natural to infer that the undetermined acids that accumulate in the serum are organic.

Hyperphosphatemia is probably likewise connected with the increased urinary phosphate excretion.[1] The inorganic phosphate is presumably derived from the destruction of organic phosphorus compounds. In the terminal stages of the disease a diminished volume of urine or anuria may exaggerate the effects of tissue destruction.

SUMMARY AND CONCLUSIONS

The concentration of proteins in the serum of monkeys diminishes during the course of yellow fever. The reduction is chiefly at the expense of the albumin fraction. In the early stages of the disease globulin rises.

In the terminal stages of the disease serum bicarbonate is usually reduced, often to a considerable extent. The reduction is due chiefly to accumulation of undetermined acids, probably organic and, to a lesser extent, of inorganic phosphate.

No characteristic alterations were found in the concentrations of base and chloride in the serum or in the urinary excretion of chloride.

PROTEOLYTIC LEUKOCYTIC ENZYME IN LEUKEMIA

A STUDY MADE BY A QUANTITATIVE METHOD

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Since the demonstration of a rather high degree of proteolytic enzyme activity in the granular polymorphonuclear leukocytes, the presence of such leukoprotease in these and other cells of the blood has been studied in various conditions,[1] as, for example, the presence of ferments in the abnormal granular and nongranular cells present in the blood in leukemia. No studies have been reported since the development of modern microchemical methods. In this paper the results of the study of proteolytic ferment by a method applicable to small amounts of blood are described.

One point of interest in the proteolytic enzyme in the abnormal cells of leukemic blood concerns the possible relation of these cells to normal lymphocytes or to the myeloblasts of the bone marrow. Small amounts of proteolytic enzymes have been described in almost all cells, and normal lymphocytes possess little more than other types of cells, while the granular polymorphonuclear leukocytes and myelocytes have a very high degree of proteolytic activity. The presence of a relatively large amount of protease in the cells in acute leukemia, therefore, would give support to the belief that the nongranular mononuclear cells present are closely allied to the marrow elements and should be considered myeloblasts or "premyeloblasts" rather than lymphocytes. Certain authors (e. g., Longcope and Donhauser[2] and Longcope and Cooke[3]) have concluded that in certain cases of acute lymphatic leukemia the cells possess proteolytic activity of a character very similar to that of the granular myelocytes and should be considered as forerunners of these cells. Other observers have not found such proteolytic activity in the

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1. Detailed reference to much of the earlier work may be found in the monograph by Noël Fiessinger, "Les ferments des leucocytes," Paris, Masson & Cie, 1923.

2. Longcope, W. T., and Donhauser, J. L.: A Study of the Proteolytic Ferments of the Large Lymphocytes in a Case of Acute Leukemia, J. Exper. Med. **10**: 617, 1908.

3. Longcope, W. T., and Cooke, J. V.: A Preliminary Note upon the Enzymes and the Leukocytes in Acute Leukemia, Proc. Path. Soc., Philadelphia **14**:104, 1911.

cells in acute leukemia, and Fiessinger [1] stated that of twenty-two such cases reported in the literature only one-half have shown proteolytic ferment.

The ideal type of blood for the estimation of leukocytic protease activity is one in which a single variety of leukocytes greatly predominates. When polymorphonuclear leukocytes are present in any considerable number, their proteolytic activity may be confusing in the interpretation of the results. In many instances the abnormal cells present in acute leukemic blood form from 80 to 90 per cent of the cells present. Any proteolytic activity present in such blood can be more readily referred to a single type of cell.

The methods used in previous studies have been similar. Blood was collected either during life or immediately after death, citrated and centrifugated. The buffy layer of leukocytes above the red corpuscles was removed by a pipet and washed with physiologic solution of sodium chloride to remove serum and its antiferment, and the leukocytes were suspended in salt solution. Protease was determined by placing a certain amount, usually 1 cc, of this leukocyte suspension in a measured quantity of coagulable protein, (e. g., heated serum water) in neutral or slightly alkaline reaction and incubating for five days at 37 C., toluene being added to prevent bacterial proteolysis. At the end of the period estimations of nonprotein nitrogen were made by the Kjeldahl method on the filtrate after removal of the undigested coagulable protein by boiling with acetic acid and magnesium sulphate buffer. The amount of protease present was estimated by obtaining the difference in nonprotein nitrogen found in the tests and that present in the uninoculated controls or in controls to which were added leukocytes heated to destroy their ferment. Some observers determined the amount of digestion by titration of the amino-nitrogen by the formaldehyde method.

AUTHOR'S METHOD

The method used in this study differs from those previously employed in that the hemoglobin of the blood is used as a substrate on which the leukocytic protease may act, and that the use of microchemical methods allows determinations to be made on 10 cc. of blood or less. This procedure permits the study of the same patient at different times, and, by the recording of the results in milligrams of digested nitrogen per hundred cubic centimeters of blood, offers a means of quantitative comparison between different samples.

At approximately the same time that a total and stained differential leukocyte count is made, a sample of venous blood is placed in a test tube with a small amount of powdered sodium oxalate to prevent clotting. The total volume having been marked on the tube, the specimen is then centrifugated and the supernatant plasma removed and discarded, care being used to avoid taking away any cells. The cells are then washed three times in 0.85 per cent salt solution to get rid of the last traces of serum, and after the last washing the same solution is added to the tube until the original volume is reached. This is well shaken to distribute the leukocytes

evenly, and 3 cc. samples are placed in large test tubes with a volumetric pipet. These are treated as follows: 1. Control.—To this tube is added 21 cc. of distilled water, 3 cc. of a 10 per cent solution of sodium tungstate and 3 cc. of two-thirds normal sulphuric acid. After shaking and filtering, the nonprotein nitrogen is determined on a sample of the filtrate and the milligrams of nonprotein nitrogen per hundred cubic centimeters calculated. 2. Protease activity in neutral medium. After the addition of 7 cc. of water and a small quantity of toluene, the tube is incubated at 37 C. for five days. Then 3 cc. of a 10 per cent solution of sodium tungstate, 3 cc. of two-thirds normal sulphuric acid and 14 cc. of water are added and the nonprotein nitrogen determination made on the filtrate.

The procedure outlined is the well known method of Folin and Wu,[4] and the determinations of nonprotein nitrogen are carried out by acid digestion and nesslerization, as usual. The results are all expressed as milligrams of nitrogen per hundred cubic centimeters of blood.

Samples of blood without leukocytes obtained by taking only the lowermost layers of cells after washing with salt solution by centrifugation show no evidence of proteolytic activity.

TABLE 1.—*Protease Activity of the Blood of Normal Children*

Case	Age	Leukocytes	Polymorphonuclears	Lymphocytes	Monocytes	Nonprotein Nitrogen, Mg. per Cent by Volume	
						Control	Protease
1	6	8,200	57	40	3	12.9	120
2	19	5,800	69	25	6	8.1	125
3	6	6,000	72	22	6	9.0	116
4	15	8,600	68	28	4	12.0	105
5	4	7,800	43	45	12	18.0	170
6	5	5,100	83	14	3	13.0	170.5
7	5	7,400	84	12	4	18.0	180
8	9	8,200	70	25	5	13.2	152

In the tables and text the results obtained in blood determinations are spoken of as "protease," but it will be understood that they really represent the result of protease activity.

RESULTS

Normal Blood.—The results of the determination of protease in the blood of a group of apparently normal persons are shown in table 1. There is some variation in different persons, but with leukocyte counts below 10,000 and the relative distribution of the cells within normal limits, the protease activity is such as to produce a figure of somewhat over 100 milligrams per cent of nonprotein nitrogen by volume. This small group will serve for comparison with later readings on abnormal leukemic bloods.

Chronic Myeloid Leukemia.—Four adult patients with typical chronic myeloid leukemia were examined through the kindness of Dr. Lawrence Thompson. All had had the disease for at least a year;

4. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**:81, 1919.

all had received roentgen therapy, and all were in a period of clinical remission when the blood was tested. Only one case in a child was studied. Only brief notes of each case will be given.

CASE 1.—H. J. S., a white man, aged 50, whose symptoms began one year previously, in August, 1929, had a total leukocyte count of 130,000, and since then had had eight weekly roentgen treatments. When seen on Feb. 4, 1930, he was in good condition; physical examination gave negative results except for a palpable spleen, and the blood showed 24,200 leukocytes.

CASE 2.—W. P., a colored man, aged 28, whose first count seven months previously had been 434,000, at the time of examination still had a much enlarged spleen; the leukocytes had decreased to 15,100. Two months later another test was made; the count was 149,000.

CASE 3.—W. T., aged 50, colored, had been under observation for two years and had had 600,000 leukocytes with from 90 to 95 per cent myelocytes. At the time the first tests were made, the count was 103,400, but five weeks later, after roentgen treatment, the leukocytes had decreased to 20,000.

TABLE 2.—*Protease Activity in the Blood of Patients with Chronic Myeloid Leukemia*

Case	Date	Age	Leukocytes	Polymorphonuclears	Myelocytes	Lymphocytes	Monocytes	Nonprotein Nitrogen, Mg. per Cent by Volume	
								Control	Protease
1	2/ 4/30	50	24,200	58	21	7	12	13.8	440
2	1/31/30	28	15,100	70.5	..	27.5	2	13.2	420
	3/14/30	..	149,000	20	70	5	5	32	1,680
3	2/ 7/30	50	103,400	46	47.5	3	3.5	22.5	280
	3/14/30	..	20,000	29	65	5	1	21	540
4	3/21/30	56	160,200	52	39	4	5	42.4	1,008
5	2/28/31	8	300,000	46	45	4	5	21	818
	3/10/31	..	23,000	77	11	8	4	15	215
	5/ 7/31	..	55,000	63.8	18.8	14.6	2.8	18.5	1,040

CASE 4.—F. S., a white man, aged 56, had had the disease about one year. Six months before the tests were made the total leukocyte count was 367,000, but following roentgen treatment the count diminished to 25,000. It had increased to 160,000 when the tests were made. The spleen was greatly enlarged.

CASE 5.—E. H., a white boy, aged 8, who had had asthenia and pallor for six months, had a greatly enlarged spleen and a leukocyte count of 300,000. A test was made at this time; another was made three weeks later, when the count had dropped to 23,000, following roentgen therapy, and a third after two months more, when the count had increased to 55,000.

The quantitative estimation of leukocytic protease on samples of blood from patients with chronic myeloid leukemia together with the blood picture at the time of the tests, is shown in table 2. In all instances, the figures obtained for protease are greatly increased as compared with those obtained from normal blood. The higher readings were usually found in patients with higher leukocyte counts, but this relation is not constant, since in case 3, the reverse is true. In one instance (case 5) the blood was first examined before treatment had

been given, when the leukocyte count was 300,000 and showed a greatly increased reading for protease (818); two weeks later, following roentgen therapy, the count had fallen to 23,000 and the protease had also decreased to 215; after two months the leukocyte count had risen only moderately (55,000), but the protease figure was 1,040. It would seem, therefore, that the amount of proteolytic ferment in the granular cells of the blood may vary somewhat.

The finding of an increase in proteolytic ferment in the blood in chronic myeloid leukemia is in accord with the reports of other workers. Since both polymorphonuclear cells and myelocytes are usually present in large numbers in this disease, it is difficult to determine the relative part played by each of these two types of cells in causing the proteolysis. However, since the protease content is high no matter which type predominates, it seems certain that both myelocytes and polymorphonuclear leukocytes contain an abundant protease in chronic myeloid leukemia.

Chronic Lymphatic Leukemia.—We have examined only a single patient with chronic lymphatic leukemia, through the kindness of Dr. H. L. Alexander.

A white man, aged 71, whose symptoms were of two years' duration, had a greatly enlarged spleen, dyspnea and weakness and had lost 40 pounds (18.1 Kg.). The leukocyte count was 505,000, practically all of which were small lymphocytes. Ten days after the first count and after roentgen therapy the count was 80,000, with 94.5 per cent small lymphocytes, 3.5 per cent polymorphonuclears and 2 per cent large mononuclears. At this time protease tests showed: control, 13.8, protease, 63, as expressed in milligrams per cent by volume of nonprotein nitrogen.

In this single observation a relatively small amount of proteolytic activity was found in the blood cells in chronic lymphatic leukemia.

Acute Leukemia.—Twenty tests for protease were made for twelve patients with acute leukemia. In every instance the disease was clinically typical, and in the five cases in which necropsy was permitted, the pathologic changes were characteristic of acute leukemia. The total number of leukocytes varied considerably in the different cases and even in the same patient, but in all there was a great predominance of pathologic nongranular mononuclear cells. Certain patients showed many of the "large lymphocytes," while in others only small numbers of such larger cells were seen, and the majority were "small lymphocytes." In every case vital and peroxydase stains were made on blood films. Sometimes very small numbers of the abnormal cells showed myelocytic granules, but almost all had no oxydase granules. The patients were of the group commonly designated as having "acute lymphatic leukemia." Brief mention of the striking features of each case is given.

CASE 1.—E. W., a white boy, 11 years old, had petechial hemorrhages in the skin, greatly enlarged liver and spleen and fever. Acute symptoms had been present for one week.

CASE 2.—D. S., a 9 year old white boy had hemorrhages in the skin and mouth, blood in the urine, fever, an enlarged spleen and cervical lymph nodes. The symptoms were first noted six weeks previously.

CASE 3.—R. C., a white boy, 3 years old, showed pallor, weakness, enlarged liver and spleen and fever. Later there were hemorrhages in the skin of the thigh and face, and at necropsy leukemic infiltration of the parenchymatous organs was found.

CASE 4.—D. R., a white boy, 9 years old, had had several attacks of fever, lasting a week or more. The spleen was enlarged, and on examination marked anemia, fever, petechiae and weakness were noted.

CASE 5.—O. McN., a white boy, 6 years old, had greatly enlarged cervical and mediastinal glands and spleen, with some cutaneous petechiae. The symptoms were almost entirely due to respiratory obstruction, and there was only slight fever. The child died a few hours after having been given roentgen therapy. Necropsy showed typical leukemic infiltration of the tissues, especially of the mediastinum.

CASE 6.—W. S., a 5 year old white boy, had a large mediastinal tumor, dyspnea and a slightly enlarged spleen. There was no hemorrhage or fever, and the symptoms were entirely those of respiratory obstruction.

CASE 7.—A. B., a colored boy, 13 years old, had complete flaccid paralysis of legs and a bladder and rectal sphincters, apparently due to leukemic tumor in, or pressing on, the spinal cord. He was not acutely ill, and except for the paralyses would not have sought medical aid.

CASE 8.—In E. S., a white woman, 30 years old, whose symptoms began four months before death, the spleen was greatly enlarged, and there was general glandular swelling. There was progressive weakness but no hemorrhage. The leukocytes numbered 30,000 two months before death, with 98 per cent lymphocytes. After intravenous treatment with antimony and potassium tartrate they had fallen to 8,000 when the first test was made. A second test was made two weeks later after the leukocytes had risen to 21,000. There was a moderate continued fever until a few days before death, when the temperature became subnormal. Marked asthenia characterized the last weeks of the disease. Necropsy revealed typical lesions of acute leukemia.

CASE 9.—D. K., a white boy, 10 years old, was acutely ill with ecchymotic and purpuric hemorrhages of the skin, enlarged cervical glands and spleen, and fever. The leukocytes numbered 200,000 on admission, but fluctuated considerably, reaching 4,800 at one time. The patient continued to be prostrated during the following month, with fever and, at times, severe abdominal pain. At necropsy a typical picture of acute leukemia was found with a ruptured appendix walled off by adhesions and an extensive terminal bronchopneumonia.

CASE 10.—H. V. L., a 9 year old white boy, had a severe angina lasting a month, with leukocytes varying from 500 to 3,600. The diagnosis was agranulocytic angina. Following twelve transfusions the patient was discharged two months after the onset, apparently well and with a normal blood picture, except for the presence of a few myelocytes. One month after discharge he was readmitted to the hospital with well marked enlargement of the lymph nodes and spleen, some petechial hemorrhages and the blood picture of acute leukemia. Later, fever, marked prostration and toxicity were accompanied by a great decrease in blood protease.

Case 11.—M. C., an 8 year old white girl, had anemia, asthenia, slight general glandular enlargement, a large spleen and fever.

Case 12.—S. B., a 4 year old white boy, had enlarged cervical and mediastinal glands when the first test was made. Fever, marked asthenia and enlarged spleen had developed when the second test was made two months later.

The single determinations of protease (cases 1 to 7) in table 3 show a variation of from 17.5 to 360 mg. in the various patients, and in one instance (case 10) the reading was 750 mg. These variations in protease were apparently not altogether dependent on the number of leukocytes, because some patients with high leukocyte counts gave low readings for protease and others with lower counts had a higher amount

Table 3.—*Blood Picture and Protease Activity in Acute Leukemia*

Case	Age	Leukocytes	Nongranular Leukocytes, per Cent	Polymorphonuclears, Number	Nonprotein Nitrogen, Mg. per Cent by Volume		Before Death	Temperature, C.
					Control	Protease		
1	11	240,000	96	9,600	13	60	2 weeks	39.6
2	9	420,000	96	16,800	18.3	165	1 day	39
3	3	18,000	86	2,500	10	38.3	1 month	39
4	9	33,000	92	3,600	10	17.5	2 months	39
5	6	320,000	92	25,600	20	340	3 days	37.4
6	6	33,000	93	2,300	12	342	2 months	37.2
7	13	103,000	97	3,100	24	360	2 months	37.6
8	30	8,200	76	2,000	6	17.5	2½ weeks	38.6
		21,000	75	5,000	12.9	60	3 days	36.4
9	10	80,000	97	2,400	9.6	67	1 month	39
		18,500	90	1,800	7.8	24	3 weeks	40
		130,000	90	13,000	15	21	1 week	39
		300,000	96	12,000	9	24	Postmortem	
10	9	37,600	78	8,200	32	750	1 month	37
		115,000	98	2,300	20	115	10 days	37.8
		163,000	98	3,300	12	26.7	3 days	39.4
11	8	10,400	84	1,700	16	38	6 weeks	38.8
		15,200	95	800	11.7	27	5 weeks	39
12	3	145,000	91	13,000	15	237	2 months	37.6
		242,000	94	14,500	10	110	5 days	39

of protease. Since it was evident that some other factor influenced the amount of protease present, an analysis was made of the clinical condition of the patients at the time of the examination of the blood. In acute leukemia there are periods, often toward the end of the disease, during which the patient shows striking clinical evidence of severe systemic disturbance, such as, for example, pyrexia, hemorrhages and intoxication. There are also periods, usually earlier in the disease, in which the clinical signs may be merely moderate anemia and asthenia with enlargement of the spleen and lymph nodes, but without toxic symptoms. The presence of febrile reaction usually indicates toxicity attributable to the leukemia. The temperature at the time the tests were made has also been tabulated, and a rather striking correspondence between the presence of fever and a low reading for blood protease is seen. This is evident, not only in various patients, but also in the

same patient when readings were made at different times. In every instance the low values were found when the patient was obviously ill and toxic.

The possible influence of the granular polymorphonuclear leukocytes present in leukemic blood on the protease content must be considered. In table 3 are tabulated the estimated numbers of these cells in each specimen tested. It is difficult to draw definite conclusions as to this point, but in certain instances, notably case 9, a rather large number of granular leukocytes was present in blood that was low in protease. This indicates that the granular cells share with the nongranular leukocytes the loss of this enzyme.

The three instances (cases 5, 6 and 7) in which a relatively high reading for blood protease was noted occurred in children without fever or other evidence of intoxication whose symptoms were due to mechanical disturbances produced by localized leukemic infiltration. Two of them had large mediastinal masses that caused marked respiratory embarrassment with cyanosis, while the third was paralyzed in both legs from a dorsal myelitis of leukemic origin.

Among the patients for whom several determinations for protease were made during the course of the disease, the one in case 10 (table 3) is also of considerable interest in illustrating the apparent relation of the clinical evidence of intoxication to the blood protease. When first tested, this boy was afebrile and free from toxic symptoms, although the lymph nodes and spleen were moderately enlarged. The protease was 750 mg. per hundred cubic centimeters of blood, similar to the high readings noted in chronic myeloid leukemia. About ten days later, a febrile attack lasting a week occurred, and another test of the blood made when the temperature had subsided somewhat showed the protease to be only 115 mg. per hundred cubic centimeters, although the total leukocyte count had increased markedly. A week later when the leukocyte count was still rising, the protease reading was only 26.7 mg. per hundred cubic centimeters. At this time the patient was very toxic, with continued high fever and hemorrhages. He died three days later.

COMMENT

From the instances cited and the others shown in table 3 it would seem that the nongranular cells in acute leukemia may contain protease ferment to a degree comparable to that found in the granular myelocytes in chronic myeloid leukemia. This high protease content of the cells, however, is demonstrable only before the patient has become febrile and toxic from the disease. When the disease reaches the stage in which toxic symptoms appear, the protease of the cells is markedly decreased or almost absent, even though the total leukocyte count is very high.

In morphologic appearance the abnormal nongranular mononucleated leukocytes present in acute leukemia resemble lymphocytes or lymphoblasts found in the lymphatic tissues, and it is common to speak of the disease as acute lymphatic leukemia. However, in the acute forms of leukemia, it is significant that the elements of the blood chiefly affected are those originating in the bone marrow, since the polymorphonuclear leukocytes, the platelets and the red corpuscles are all constantly decreased. The abnormal nongranular cells that appear in the blood, by their high content of protease, seem also biologically closely related to bone marrow tissue, as the only other cells known to be characterized by such abundant protease are myelocytes and polymorphonuclear leukocytes. These facts make it much more probable that the abnormal cells in acute leukemia have their origin in the bone marrow, and that the disease should be considered primarily one affecting the hematopoietic marrow tissues rather than the lymphatic tissues.

It is true that the lymphatic glands may become enlarged in acute leukemia, although this enlargement is often relatively insignificant and tends to occur late in the disease. Microscopically, the cells found infiltrating the lymph glands are apparently identical with the abnormal nongranular mononuclear cells of the blood. If these cells originate in the lymph glands and subsequently pass into the blood they should be considered lymphocytes. However, there is no evidence that these infiltrations of the lymph nodes are not secondary, with the cells originating in the marrow and passing to the lymph nodes by way of the blood stream like similar cell infiltrations of the liver, spleen and kidneys, which are rather constant in the disease. The presence of considerable amounts of proteolytic ferment in these cells is evidence that strongly suggests that their origin is myeloid rather than lymphoid. Their relation to myeloblasts or other marrow cells and the nature of the changes that lead to the loss of protease activity during the course of the disease can only be conjectured.

Since there is some question about the justification for considering the abnormal nongranular leukocytes of the acute form of leukemia as lymphocytes, it would seem proper to designate the disease as "acute leukemia" rather than "acute lymphatic leukemia."

SUMMARY

A method for quantitative determination of protease activity applicable to small amounts of blood has been used in studying patients with leukemia. The results are expressed in milligrams of nonprotein nitrogen per hundred cubic centimeters.

In chronic myeloid leukemia the blood protease was found to be considerably increased. Although the readings usually varied in direct

relation to the leukocyte count, there were certain exceptions that indicated that the protease activity of these cells might vary under certain conditions.

In the single case of chronic lymphoid leukemia studied the blood protease was decreased.

In acute lymphatic leukemia, both high and low values for blood protease were found. An analysis of twenty determinations made on twelve patients indicates that the nongranular leukocytes in acute leukemia may have a rather high protease activity, comparable to that of the granular cells, but that this protease content becomes markedly decreased in the late stages of the disease. A similar decrease in protease activity of these cells accompanies toxic periods, shown by fever and prostration, which may occur in the course of the illness.

The fact that the abnormal nongranular leukocytes of acute leukemia may contain abundant protease, even though their enzyme content may be decreased in certain stages of the disease, is interpreted as evidence that these cells should be considered more closely related to the granular myelocytes than to the true lymphocytes, and that their origin is in the bone marrow and not in the lymphatic system. It seems probable that the essential site of disturbance in acute ("lymphatic") leukemia is the bone marrow and not the lymphatic system.

CHRONIC GASTRIC ULCERS

HISTOLOGIC OBSERVATIONS ON THE FACTORS UNDERLYING THE HEALING OF LESIONS PRODUCED EXPERIMENTALLY IN RABBITS

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In 1928, I reported cytologic observations on the regenerative phenomena that followed the surgical removal of large areas of gastric mucosa in the dog.[1] These areas represented experimental acute gastric ulcers. Such acute lesions healed rapidly, and the process was followed from the earliest changes to complete restoration with newly differentiated epithelium. The type of cell responsible for regeneration of this epithelium was identified and was found to be the so-called foveolar cell which forms the foveolae of normal gastric glands.

Similar work was attempted in the rabbit, but it was evident that, with the technic employed, the process in this animal was somewhat different from that observed in the dog. The acute lesions in the rabbit instead of healing tended to become chronic ulcers in a large percentage of the experiments. Reports on a few of these ulcers were included in my previous article. The successful production of such chronic lesions gave an opportunity for study of the healing processes in experimental chronic gastric ulcers. I have since produced another series of chronic ulcers of this kind in which the lesions varied in age from 3 months to more than 2 years; they represented all stages from ulcers with practically no healing to almost completely healed lesions. A cytologic study of this series of experimental chronic gastric ulcers in rabbits constitutes the present report.

MATERIAL AND METHODS

The animals used were large rabbits (mostly Flemish giants) weighing between 4 and 5 Kg. An effort was made to use old rabbits, and all of them were at least adults. About 60 per cent were females. After their recovery from the operation to be described, some of these animals were used for breeding purposes without any apparent influence on the experiments.

Submitted for publication, July 31, 1931.

From the Department of Anatomy, Northwestern University Medical School, and the Department of Medicine, University of Chicago.

1. Ferguson, A. N.: A Cytological Study of the Regeneration of Gastric Glands Following the Experimental Removal of Large Areas of Mucosa, Am. J. Anat. **42**:403, 1928.

As in previous experiments reported, the animals were operated on on a full stomach, and food was left in their cages so that they might eat as soon as they chose after operation. Their diet consisted uniformly of carrots, oats and hay and, frequently, lettuce. All operations were done with aseptic technic under ether anesthesia. An incision was made in the anterior wall of the stomach at about the junction of the body of the stomach with the pyloric portion. This original incision extended through the serosal and muscular coats down to the submucosa and underlying mucosa but not through these layers. One edge of the muscular coat was then lifted up, and under-cutting was performed through the submucosa for about 2 cm. A circular area of the exposed mucosa, at least 1.5 cm. in diameter, was then excised by means of scissors, after which the original incision in the muscular coat was sewed together. This left the line of suture in one side of the floor of the ulcer.

The primary mortality from these operations was rather high, death being due to perforation of the stomach at the site of the ulcer, perigastric abscess or incidental infections. Animals that survived were allowed to live for varying periods. A series of lesions (eighteen) was obtained ranging in age from 95 days to 2 years, 1 month and 17 days. The usual length of life of a normal Flemish giant rabbit is about five years. Therefore, a lesion of over two years' duration occupies a large part of the animal's life cycle.

MICROSCOPIC TECHNIC

All of the areas of ulcer were pinned on cork and fixed in a formaldehyde-Zenker solution, after which appropriate blocks were embedded in paraffin. Sections were cut serially 5 microns in thickness. The following general and differential stains were employed: (1) Ehrlich's hematoxylin and eosin, (2) Mallory's connective tissue stain, (3) iron hematoxylin and muci-carmine and (4) Bensley's tricolor stain (Klein [2]).

MACROSCOPIC OBSERVATIONS

When the animals were opened for removal of the experimental tissues, surprisingly few adhesions were found in the region of uncomplicated ulcers. In some cases a collection of fatty tissue, sometimes as large as an almond, was adherent to the stomach wall, but often there was no external evidence of ulcer whatever. Especially was this true of the older specimens.

Usually the site of the ulcer was recognized without difficulty, and chronic ulcers were plainly evident (fig. 1, *A* and *B*). In partially healed lesions the extent of the regenerated epithelium could be grossly estimated. This varied from only a small amount of epithelium extending centrally onto the floor of the ulcer in some specimens (fig. 1, *C*) to a complete covering in others (fig. 2). Gradations between these extremes were collected (fig. 1, *D*). Almost completely healed ulcers were recognized by the raylike arrangement of small rugae about them, by the roughened and slightly depressed surface of the regenerated epithelium of the mucosa and by their whitish appearance in contrast

2. Klein, R. L.: The Granule Cells of Paneth, Am. J. Anat. **5**:315, 1906.

to the surrounding darker mucosa. The whole had a distinctly puckered appearance. In a few cases healing was so complete that the areas of ulcer could not be recognized definitely, and such specimens were discarded as completely healed lesions unrecognizable grossly.

The mucosa just peripheral to the margin of each ulcer, especially in the more chronic ones, was thrown up into many small irregular

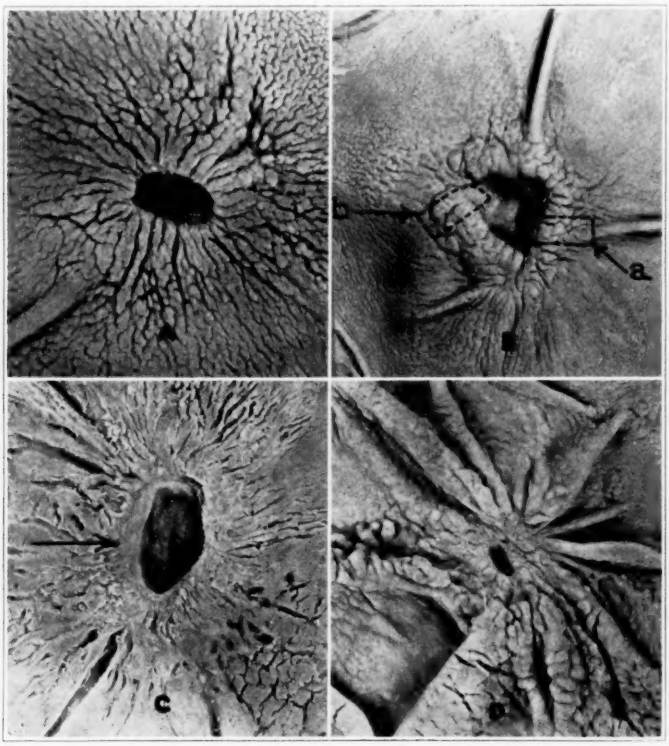

Fig. 1.—*A*, surface view of a chronic ulcer 3 months and 13 days old. There is no apparent healing although it is only about half the size of the original lesion made surgically. Approximately natural size. *B*, surface view of a chronic ulcer 2 years, 1 month and 17 days old. It is about one half the size of the original lesion. Approximately natural size. *C*, surface view of an ulcer 9 months and 9 days old. Regeneration of epithelium can be seen in the crescent indicated by the arrow. Approximately natural size. *D*, surface view of an ulcer 10 months and 28 days old. Considerable healing has occurred, and only a small crater remains. Approximately natural size.

elevations instead of the usual smooth membrane (fig. 1, *B*). This zone, extending about 5 mm. peripherally, suggested a piling up of the epithelium in this region. In several ulcers the linen suture string

placed in one side of the base at operation protruded from the crater of the ulcer (fig. 2). In some specimens this extended as a loop from one side of the crater to the other.

A study of the table brings out two points: first, even though an ulcer tends to remain chronic (for example, 24), its size as compared to the original lesion decreases considerably. Second, by itself, the age factor is relatively unimportant. Some lesions tend to heal in a few months while others continue their tendency toward chronicity even after two years. The essential factor determining the rate of healing is apparently, as will be seen later, the relative balance existing between the destructive and the reparative factors.

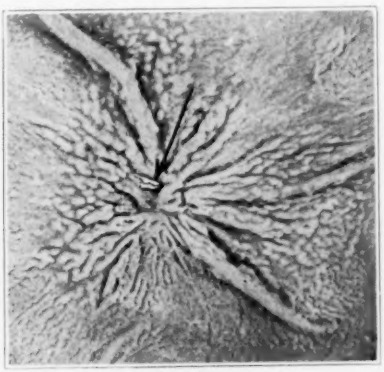

Fig. 2.—Surface view of an ulcer 9 months and 9 days old. It is almost completely healed. The suture string, indicated by an arrow, can be seen protruding. Approximately natural size.

Age and Size of Ulcers

Number of the Animal	Age of the Ulcer in Days	Size of the Ulcer in Millimeters
19	95	3 by 3
2	100	12 by 14
24	105	4 by 7
18	120	Edges approximated; almost healed
11	164	1 by 1
20	213	3 by 3
17	224	Edges approximated; almost healed
25	283	6 by 11
9	285	Edges approximated; almost healed
10	288	Edges approximated; almost healed
3	296	1 by 2
23	333	1.5 by 3
21	401	Edges approxmiated; almost healed
6	472	Edges approximated; almost healed
1	515	Edges approximated; almost healed
26	562	1 by 1
5	734	0.5 by 2
7	778	5 by 7

MICROSCOPIC OBSERVATIONS

The ulcers produced in these experiments apparently became chronic soon after they were formed, and their subsequent course depended on whether they tended toward further chronicity or toward healing. All, however, showed attempts at healing and repair. The amount of healing varied a great deal in the different specimens, some tending to remain chronic, others being partly healed and still others almost completely repaired. A description of the microscopic features of any one ulcer, would depend, therefore, on the amount of healing effected and

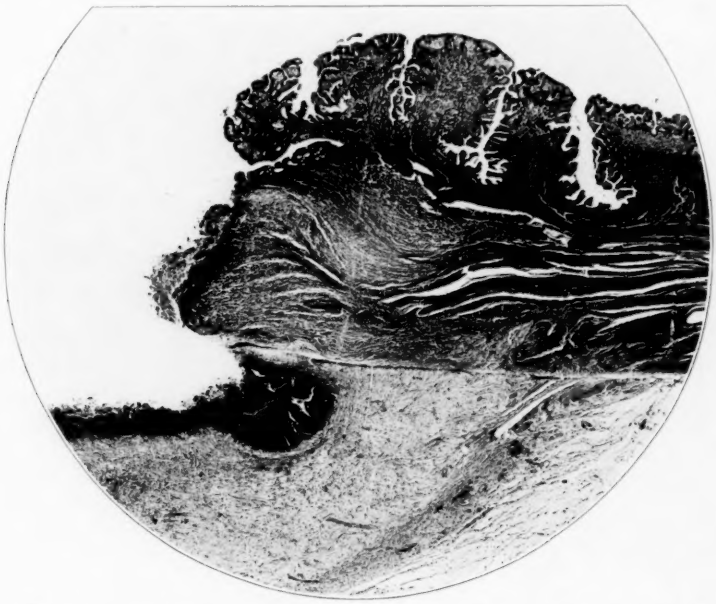

Fig. 3.—A section through the cardiac side of the chronic ulcer shown in figure 1, *B* (taken from region indicated by arrow *A*), showing the epithelial folds described and the undercutting of the base at the margin. The densely stained superficial material on the floor of the crater is the necrotic layer of the base. Magnification, × 18.

the character of the base, irrespective of its age. A conception of the microscopic conditions prevailing can be easiest gained by a description first of an ulcer with a tendency toward chronicity, then of a lesion almost completely healed and, finally, of intermediate stages between these extremes.

Typical Chronic Ulcer.—For the description of an ulcer with chronicity, the one obtained from rabbit 7 was selected (figs. 1 *B*, 3, 4,

5 and 8). This lesion was made surgically two years, one month and seventeen days before the animal was killed and the ulcer examined. As one approached the margin of the ulcer, from areas of normal stomach tissue, all layers of the stomach were gradually increased in thickness. This condition began approximately 5 mm. from the margin of the ulcer. Examination of the mucosal layer showed that it was composed of folds (fig. 3) instead of the usual rather regular glandular arrangement. The largest folds bordered the crater of the ulcer and tended to lean toward it. The epithelium between these folds reached down in many places almost to the muscularis mucosae. Parietal and

Fig. 4.—A section through the pyloric side of the chronic ulcer shown in figure 1, *B* (taken from region indicated by arrow *B*). The marginal epithelium extends out onto the surface for a short distance. There is very little undercutting of the margin at this location. Magnification, × 90.

serous chief cells gradually disappeared as the margin was approached, so that the last folds were composed entirely of foveolar cells. Finally only a narrow rim of flattened cells extended for the distance of a few cell breadths on the surface of the ulcer (figs. 4 and 5). It has previously been shown that these foveolar cells are the ones responsible for regeneration of the mucosa.[1] Connective tissue elements between the glands forming this epithelium were increased over the usual amount and there was some infiltration of leukocytes, especially eosinophils and plasma cells.

As the deeper layers of the stomach wall increased in thickness, approaching the margin of the ulcer, the submucosa was correspondingly decreased until finally it disappeared altogether. At the same time, smooth muscle fibers diminished in number in the tunica muscularis, and a compensatory amount of connective tissue appeared until, within about 1.5 mm. of the margin, the wall was composed entirely of a mass of connective tissue which continued into the base of the ulcer (fig. 3).

The base or floor of the ulcer was composed of two main layers covered externally by serosa (fig. 3). The first or superficial one may

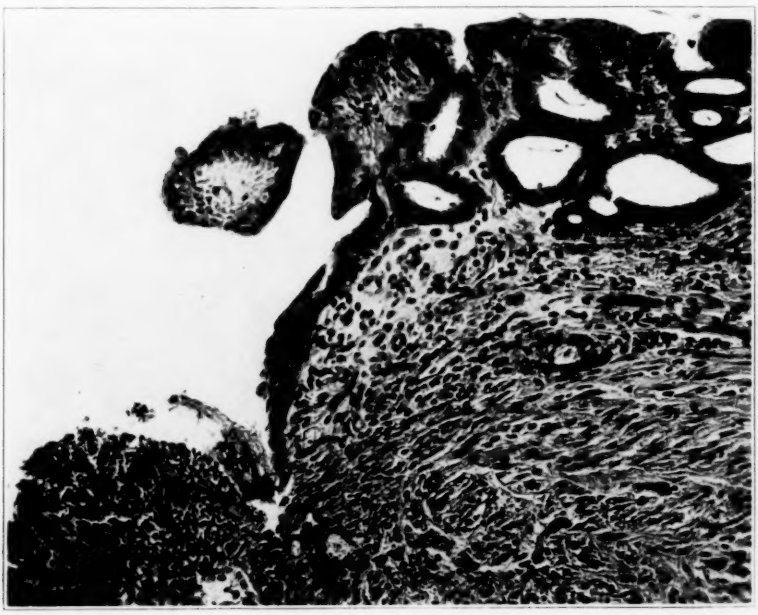

Fig. 5.—A higher magnification of part of the section shown in figure 3, showing the epithelial cells extending furthest onto the floor of the ulcer. Magnification, × 150.

be called the necrotic layer. It formed the floor proper and occupied about one fifth of the thickness of the base. It was composed of cell débris and nuclei that stained darkly with hematoxylin. Many of these latter components were lobulated, and it is assumed that they had their origin in disintegrating leukocytes. This surface layer changed rather abruptly into the underlying or connective tissue layer which was composed of a mass of fibroblasts and young connective tissue cells with many blood vessels. The fibroblasts had rounded nuclei and large amounts of cytoplasm that extended into branched processes. Apparently

these cells were exhibiting great proliferative activity. The deeper part of this layer was somewhat more dense than the stratum just beneath the necrotic layer. It also contained some collagenous fibers arranged indiscriminately. Many leukocytes, especially eosinophils, were infiltrated throughout this tissue. These were most numerous just beneath the necrotic layer. Considerable undercutting into the base was present at the margin, which was accentuated by some overgrowth

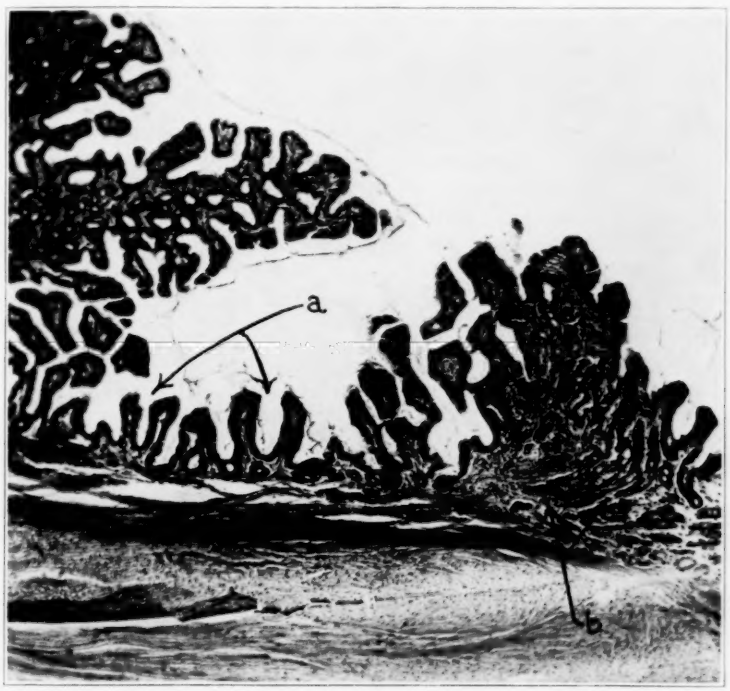

Fig. 6.—Section from an ulcer almost healed that is completely covered by regenerated epithelium. Note the early gland anlages indicated by arrow *A*. Some specialized cells (parietal and serous chief cells) are present in the region indicated by arrow *B*. Magnification, × 75.

of marginal tissue. This undercutting occurred just centrad to the point where the last epithelial cells extended out onto the surface of the ulcer, and was more pronounced on the cardiac side of the lesion (fig. 3).

Typical Ulcer Almost Healed.—As previously stated it was not possible to recognize a completely healed lesion positively; therefore a description of one almost healed is given. As a basis for this description the lesion from animal 9 was selected (fig. 6). This lesion was examined

nine months and eleven days after it was produced surgically. There was no appreciable thickening of the total stomach wall, but the mucosa was about two thirds of the normal thickness, while the base was slightly thicker than the usual muscular coat. The mucosa was thrown up into small folds. Most of the epithelium was composed of foveolar and mucous chief cells, but some specialized cells, parietal and chief serous, were seen. These were arranged in small irregular glands with some connective tissue separating them. Apparently regeneration of the mucosa had occurred similar to that described for the dog [1] except that the process was slower. The underlying wall of the stomach was composed of a mass of connective tissue intermingled with bundles of smooth muscle fibers. There was no regular arrangement of these tissues whatsoever, and the whole had a rather compact structure. The mucous membrane rested directly on this tissue. There was no distinct muscularis mucosae and no submucosa. All the cell types present in both the mucosa and the deeper layers of the normal stomach were therefore represented in this healed specimen, but their arrangement was modified as noted. Healing, therefore, may be practically complete. Whether or not such a healed ulcer is a vulnerable area in the stomach that may give rise to another ulcer is questionable.

Specimens Intermediate Between Chronic and Healed Lesions.—The description of any ulcer intermediate between the two described above depends on two factors. One is the amount of healing, as represented by regenerated epithelium extending out onto the surface of the ulcer, while the other concerns changes in the base. Both of these are a measure of the reparative processes, if any exist, in the ulcer. In some specimens these processes were small in amount; in others considerable healing had occurred, and in several it was almost complete. In a few cases one gets the impression that healing proceeded up to a certain point and then for some unknown reason stopped. This was especially true in the ulcer obtained from rabbit 23 (fig. 7), which was ten months and twenty-eight days old. Here in the angle of the undercutting in the base, many yeast cells were seen, and they may have been the factor responsible for the apparent delay of healing in this instance.

One negative observation in these experiments is of importance. In the original report [1] a few small, isolated alveoli were recorded as being seen rather deep in the floor and base of the twenty-eight and forty day ulcers in the rabbit. These alveoli were formed by a single layer of undifferentiated cuboidal cells, and some could be traced to the regenerating epithelium at the margin. The possibility of a malignant growth developing from such alveoli was considered. In fact, the present work was begun for the purpose of testing whether an experimental carcinoma of the stomach would result. The hypothesis was that

foveolar cells (which are the least differentiated cells in the gastric mucosa), in their repeated unsuccessful attempts to repair the ulcer defect may continue to revert to a less differentiated cell type until they finally break away from cell control and become malignant. The results did not support such a hypothesis. Even though the chronic ulcers occupied a large part of the life cycle of the animal used (the rabbit), in some cases (two fifths of the total number), there was no evidence of carcinoma in any of the specimens.

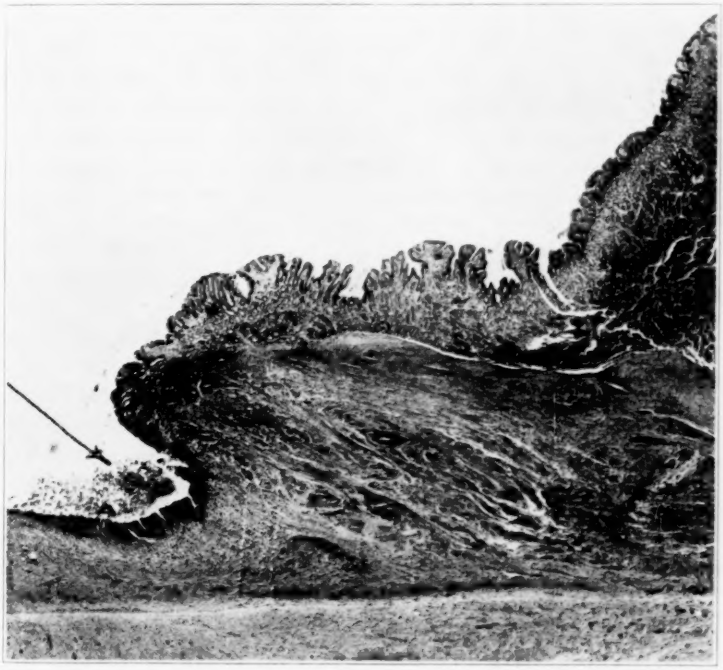

Fig. 7.—Section of the lesion obtained from animal 23. Considerable regeneration of the mucosa has occurred, but healing has apparently stopped. Yeast cells were present in the location indicated by the arrow. Magnification, × 25.

COMMENT

A general consideration of all observations shows that there is wide variation in the healing of the experimental lesions produced in the gastric mucosa in different rabbits. In some the ulcer tends to remain chronic, while in others it tends to heal. This proved true even though the animals were fed on identical diets and kept under the same conditions. The time factor, then, is relatively unimportant in considering

any given ulcer. On the contrary, the really important features are the amount of healing, if any, or the degree of chronicity.

Another significant fact is that even though an ulcer remains chronic and without any regeneration of epithelium over its base, it nevertheless tends to decrease in size when destructive processes are not too great. This may be due to three factors. First, there is a mass proliferation of epithelium back of the margin which tends to crowd the entire margin out over the base. This is evidenced both by the many small folds seen macroscopically back of the margin and by the number of cells and mitotic figures seen microscopically. A second possibility is the loss of substance from the floor of the base. It is conceivable that, as substance is lost from the floor of the ulcer by destructive processes playing on it, there is a compensatory shrinking of the base. This interpretation is supported by the appearance of the suture string in the crater of the ulcer. Originally this string was placed just beneath the serosa at operation. Considerable loss of substance must have occurred, therefore, for this suture to appear in the crater of the ulcer. Still another possibility for this decrease in size of the ulcers is the contraction of scar tissue in the base, but this probably plays a minor rôle. Most of the connective tissue in the base of these experimental chronic ulcers is young and has very little collagen. Just how far reduction in the size of a chronic ulcer may go is problematic.

It was noted that the connective tissue in the base of these chronic ulcers was relatively young and contained many blood vessels. Apparently, destructive forces playing on the floor of the base cause such a rapid loss of substance that the underlying connective tissue does not have time to reach maturity. The large number of blood vessels indicates good vascularity and speaks against poor blood supply as the cause of chronicity. Destructive forces, to be enumerated presently, are believed to be the chief factors in chronicity.

It is evident that there are two main structures involved in the healing of a chronic ulcer, namely, the marginal epithelium and the base. The epithelium attempts to cover the base in two ways, i. e., by mass proliferation, which crowds the entire margin out onto the floor of the base, and by the regeneration, from the most central marginal cells, of new epithelium that creeps out over the floor. The epithelial cells composing this margin are of a constant type. They are all foveolar cells, similar to those that form the foveolae of normal gastric glands. Thus the cells responsible for the regeneration of epithelium in acute ulcers [1] are also responsible for this regeneration in chronic ulcers. It seems that the foveolar cells stand at the margin of the ulcer waiting for an opportunity to extend out over the floor and cover it.

The work of Dragstedt and Vaughn [3] supports the interpretation just advanced. These investigators sutured various organs into the wall of the stomach and exposed them to the action of gastric juice. They found that new gastric mucosal epithelium regenerated over such tissues as parenchyma of the spleen and concluded that such immature epithelial cells are quite resistant to gastric corrosion. The indications are that chronicity in ulcers is not due to a deficiency in the epithelium.

The other important structure, from the standpoint of healing, is the base. The present work supports the idea that a continual struggle occurs in the base between destructive forces (to be discussed later) on the one hand and reparative process on the other. When the destructive forces exceed the reparative ones there is loss of substance from the floor of the base. This is evidenced not only by the necrotic layer that forms the floor and by the frequent appearance of the suture string (placed near the serosa at operation), but by the occasional occurrence of perforation. In this condition of conflict so much necrotic material and débris are present on the surface of the ulcer that it is impossible for the waiting epithelium at the margin to grow out onto the floor. If, however, reparative processes are in the ascendancy, a suitable floor is provided so that epithelium at the margin is able to gain a foothold and advance the regeneration. The extent of healing depends on the amount of these reparative processes. When they are in sufficient excess, complete healing takes place.

The destructive forces acting on the base may be mechanical (such as coarse food or foreign bodies [suture string]), chemical (such as acid and the gastric ferments) or infectious processes. Investigation of the nature and relative importance of these has been begun.

A review of the literature reveals that Crohn [4] made a postulate that is supported by the present work. He said: "It may be safely assumed that the characteristic features of an ulcer are the result of two opposing influences (1) The causative destructive factors which continued to operate and (2) The protective reaction of the organism, the reparative factors. The histologic appearance of any individual ulcer, therefore, would seem to depend on which of these two influences is in the ascendant at the time the lesion is secured." Caylor [5] brought forth this same idea.

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3. Dragstedt, L. R., and Vaughn, A. M.: Gastric Ulcer Studies, Arch. Surg. **8**:791 (May) 1924.

4. Crohn, B. B.; Weiskopf, Samuel, and Aschner, P. W.: The Life Cycle of Peptic Ulcer, Arch. Int. Med. **35**:405 (April) 1925.

5. Caylor, H. D.: The Healing of Gastric Ulcer in Man, Ann. Surg. **83**:350, 1926.

Some experimentation also has been done that supports this idea of destructive versus reparative processes. Mann [6] found that healing of ulcers occurred after diversion of the gastric contents by closure of the pylorus and a gastro-enterostomy. This prevented the gastric contents (mechanical and chemical factors) from passing over the surface of the ulcer. In the process of healing, new granulation tissue formed in the base and subsequently epithelium grew out to cover it. Fauley and Ivy,[7] using my method of production of chronic ulcers, showed that a bland diet favors the healing process in the rabbits.

Several other observations have been made on the factors that delay or hasten healing, but most of this work has been done on acute lesions. Friedman and Hamburger [8] found that retention of acid secretion in the stomach for an abnormal period delayed healing. Conversely, Dragstedt and Vaughn [3] showed that the administration of alkalies aided healing. Morton [9] showed that diversion of the duodenal contents delayed the healing of gastric lesions. He altered the gastro-intestinal tract surgically so that regurgitation of duodenal contents into the stomach could not occur. The contents were allowed to empty into the intestines several centimeters below the usual site. Hughson [10] expressed the belief that a gastric ulcer near the pylorus causes reflex pylorospasm and that healing is aided by a splitting of the pylorus. Ivy [11] found that mechanical irritation, such as rubbing the exposed ulcer with bread crumbs, delayed healing. It is probable that some of the factors mentioned are also of importance in the healing of chronic ulcers.

SUMMARY

1. A method for the consistent production of experimental chronic gastric ulcers in rabbits is described.

2. A variation in the reparative powers of rabbits with chronic ulcers was noted. In some the ulcers tended to remain chronic while in others various degrees of healing, even resulting in complete repair, occurred.

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6. Mann, F. C.: Chemical and Mechanical Factors in the Experimentally Produced Peptic Ulcer, Tr. A. Am. Physicians **42**:224, 1927.

7. Fauley, G. B., and Ivy, A. C.: Experimental Gastric Ulcer: The Effect of the Consistency of the Diet on Healing, Arch. Int. Med. **46**:524 (Sept.) 1930.

8. Friedman, J. C., and Hamburger, W. W.: Experimental Chronic Gastric Ulcer, J. A. M. A. **63**:380 (Aug. 1) 1914.

9. Morton, C. B.: Observations on Peptic Ulcer, Ann. Surg. **87**:401, 1928.

10. Hughson, W.: Relation of Pylorus to Duration of Experimental Gastric Ulcer, Arch. Surg. **15**:66 (July) 1927.

11. Ivy, A. C.: Studies on Gastric and Duodenal Ulcers, J. A. M. A. **75**:1540 (Dec. 4) 1920.

3. Experimental chronic ulcers in rabbits tend to decrease in size, when destructive processes are not too great, even though there is no regeneration of epithelium over the surface of the ulcer.

4. Epithelium forming the margin of a chronic ulcer is composed entirely of foveolar cells. These cells constantly attempt to regenerate and repair the ulcer defect, and are successful when a floor suitable for their growth is formed. Foveolar cells are responsible for regeneration of the epithelium in both acute and chronic experimental ulcers.

5. The essential factor that delays healing and produces chronicity in ulcers is that of destructive forces acting on the floor of the base. There is a constant struggle between these destructive forces and reparative processes. The outcome depends on which one is in excess and gains control.

CORRELATION OF LINGUAL CHANGES WITH OTHER CLINICAL DATA

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The study of the tongue is as ancient as the practice of medicine. Hippocrates [1] has left many notes of his observations of this organ. Repeated references in his clinical descriptions of the fevers are found to the tongue that is dry or parched. Sooty and dry is another qualifying expression used by Hippocrates relative to the tongue. Again he says, "If in a winter fever, the tongue be rough, and if there be swooning, it is likely to be the remission of the fever." An even more characteristic example is to be found in the following quotation: "When there is a chronic sore on the side of the tongue the surgeon should examine whether it be not occasioned by the sharp edge of a tooth." Through the generations down to the modern period of medicine the study of the tongue has been held in high esteem not only by the medical profession, but by the laity as well. Indeed, when the clinical investigation was limited to an anamnesis, general inspection and palpation, palpation of the pulse and the examination of the physical characteristics of the dejecta, a disproportionate significance might well be placed on the observations of the tongue. Lewis [2] expressed a colloquialism in "Raw red tongue, raw red gut." Throughout the world alterations in the appearance of the tongue are related in the lay mind to disorders of the gastro-intestinal tract. Indeed it has frequently been considered the mirror of the stomach.

Granting the abnormalities of the tongue that attend gastro-intestinal diseases, infectious diseases, exanthems and many other diseases both local and remote, there remains a well defined group of apparent deficiency diseases which arrests the attention by reason of the similarity of their concurrent involvement of the tongue. Glossitis was reported in the severe anemias of pregnancy by Larrabee.[3] Isaacs,

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1. The Genuine Works of Hippocrates, translated by F. Wood, New York, William Wood & Company.

2. Lewis, G. E.: The Smooth Tongue: A Study in Deficiency Disease, Practitioner **125**:749 (Dec.) 1930.

3. Larrabee, R. C.: The Severe Anemia of Pregnancy and the Puerperium, Am. J. M. Sc. **170**:371 (Sept.) 1925.

Sturgis and Smith [4] noted smoothing of the tongue in the anemia caused by infestation with Dibothryocephalus latus. Keefer and Yang [5] determined a glossitis in patients suffering from the anemia of undernutrition attendant on dysentery. Recovery from the dysentery and anemia was marked by coincident improvement in the glossitis. Witts [6] described an active glossitis with denudation to actual baldness in simple achlorhydric anemia. Lewis [2] reported a case in which pyloroplasty was complicated by peritonitis and obstruction, and in which a jejunostomy was succeeded by a red tongue with low papillae. To this group may be added the more widely recognized conditions, sprue, pellagra and pernicious anemia, all of which are attended in a high percentage of instances by frank glossitis, advancing in some cases to complete baldness of the tongue. The factors common to all of these conditions, which may represent deficiencies of one type or other, are the inflammation and atrophy of the tongue.

Particular attention is directed to the association of the atrophic tongue with the pernicious and the achlorhydric forms of anemia by reason of the availability of such material for study. In the case of pernicious anemia, Hunter [7] credited Barclay [8] with the first description of the condition. The original text is not accessible, but Hunter's quotation [7] rather clearly defines Barclay's patient as suffering from the anemia of pregnancy (puerperium): "Soon after her confinement she had a sore mouth, for which she applied for advice, and was ordered to leave off beer and meat, and confine hereself to slops. To this she attributed her debility, as she had continued to suckle her child, and had never been able to get up her strength properly."

Likewise, in Müller's monograph [9] at least four of the five anemic subjects reported to have suffered from stomatitis were clearly dependent on pregnancy for the inception of their trouble. His analysis of this situation is, however, worthy of preservation:

> In some cases (20, 23, 29, 38, 39) there was a peculiar stomatitis. There formed in the oral cavity, especially on the tongue and on the bottom of the tongue, small ulcers of whitish color, about the size of a lentil. The same were very resistant

4. Isaacs, R.; Sturgis, C. C., and Smith, M.: Tapeworm Anemia, Arch. Int. Med. **42**: 313 (Sept.) 1928.

5. Keefer, C. S., and Yang, C. S.: Anemia of Undernutrition, Nat. M. J. China **15**:701 (Dec.) 1929.

6. Witts, L. J.: Simple Achlorhydric Anemia, Guy's Hosp. Rep. **80**:253 (July) 1930.

7. Hunter, William: Severest Anaemias, New York, The Macmillan Company, 1909, vol. 1.

8. Barclay: M. Times, 1851, p. 480; quoted by Hunter (footnote 7).

9. Müller, H.: Die progressive perniciose Anämie, Zurich, 1877, p. 207.

to local treatment, disappearing spontaneously or with temporary improvement of the general condition, and returning again without special cause to the great torment of the patient, since they pained greatly and rendered eating difficult, now and then almost impossible. It seems logical to assume that this stomatitis stood in relationship to the alteration of the blood, that certain chemical substances are in the blood which in greater quantity mix with the secretions of the mouth and through an irritating influence bring about the ulcers in a manner similar to that described by von Mosler for other illnesses from the parotid secretion.

Before Addison's description of the anemia that bears his name (1855), an American clinician, Dawson,[10] in an exhaustive article on the tongue in diagnosis, called attention to the appearance of this structure in chlorosis. He noted that it was pallid and showed tumefactions and occasional fissures. Furthermore, it was not usually coated. "In many cases the papillae have been almost entirely obliterated, giving to the organ an unusually smooth appearance." Since methods of laboratory and clinical precision were not available at that period (1846), it is possible that Dawson actually first recognized the tongue of pernicious anemia. Further force is added to this claim for recognition by the succeeding passage, which states that a similar appearance of the tongue occurs in a nervous diathesis.

Laache[11] gave the most accurate description of any of the earlier writers on this point: "Die Zunge blank, Glatt, ohne Andeutung zu Papillen; dieselbe ist an den Randern gewissermassen ausgehackt, und hier hat er ein wunder Gefühl." (The tongue smooth, clean, without sign of papillae; it is to some extent hacked on the margins, and here it has a sore feeling.) The relationship of the tongue to the intestinal manifestations of pernicious anemia was appreciated by Laache in a description of the mouth as skinless (hautlos) at the time of diarrheal manifestations. This point was further stressed by McPhedran,[12] who reported soreness of the mouth and excoriation of the anus in a patient with pernicious anemia coincident with diarrhea and a highly colored urine. Hunter[13] recorded a case of pernicious anemia in which the soreness of the tongue "seemed to spread down into the stomach and right through him."

To Hunter[14] the medical profession is indebted for the most complete study of the glossitis of pernicious anemia. He described the

10. Dawson, J.: Thoughts on the Tongue as an Element in Diagnosis, West. J. Med. & Surg. **6**:277 (Oct.) 1846.

11. Laache, S.: Die Anämie, Christiania, Malling, 1883, p. 144.

12. McPhedran, A.: Pernicious Anemia: With a Report of Five Cases, M. News **57**:362 (Oct. 11) 1890.

13. Hunter, William: Further Observations on Pernicious Anemia (Seven Cases): A Chronic Infective Disease; Its Relation to Infection from the Mouth and Stomach; Suggested Serum Treatment, Lancet **1**:221, 296 and 371, 1900.

14. Hunter (footnotes 8 and 13).

injection of the margins of the tongue with the tiny vesicles that appeared in patches and shifted in location from time to time. Succeeding this inflammatory stage came denudation and atrophy of the intervening mucosa. At times these manifestations subsided with the advance of the anemia, and in other instances only after atrophy of the papillae was complete. He pointed out: (1) "that the glossitic changes were of varying intensity in different cases—sometimes of an acute and subacute inflammatory character, followed later by degenerative and atrophic changes, at other times more chronic; and (2) that they are closely similar to those met with in the mucosa of the stomach, and, less commonly, of the intestine." Particularly was Hunter impressed by the distinctive mode of onset, persistence and periodicity of the glossitis. To him the tongue changes constituted the most important signpost in the study of the progress of pernicious anemia, because of their accessibility. The histologic changes described by him included the round cell exudation and proliferation in the tissues and walls of the blood vessels of the tongue. Atrophy of the epithelium succeeds necrotic and mucoid degeneration. Eventually the epithelial layer is replaced by a thin fibrous sheet, and this process of scarring dips down into the muscular tissues of the tongue, indicating the depth to which the inflammatory process has preceded. Cultural studies revealed streptococci in these deeper layers. This work was confirmed by Schneider and Carey.[15]

With the advent of liver therapy and the establishment of its specific virtue in the induction of blood remissions, there came a renewed interest in the glossitis of pernicious anemia. Minot and Murphy[16] reported on this detail as follows:

> The distressing tongue symptoms so characteristic of pernicious anemia usually vanished soon after liver was taken. Vesicles on the edges and tip of the tongue disappeared, as did the red-streaked, raw or beefy appearance. The sense of rawness or pain in the esophagus subsided. In a few patients who had a pronounced disorder of the central nervous system, the disappearance of the tongue symptoms and signs was less rapid. In none of the patients who have continued the diet well have the tongue symptoms either persisted or returned. Within a few months the tongue has usually lost its shiny appearance entirely and has appeared normal.

Ample confirmation for this conclusion is available in a vast literature, which has grown about the subject of liver therapy in pernicious

15. Schneider, J. P., and Carey, J. B.: The Nature of the Glossitis in Pernicious Anemia, Minnesota Med. **10**:214 (April) 1927.

16. Minot, G. R., and Murphy, W. P.: A Diet Rich in Liver in the Treatment of Pernicious Anemia, J. A. M. A. **89**:759 (Sept. 3) 1927.

anemia.[17] Huston [18] reported a uniform improvement in the condition of the mouth, "but cases having true atrophy of the tongue show no improvement in this respect." Beyond a doubt this point of view is erroneous since a number of patients with the completely bald tongue of pernicious anemia have shown a complete return to gross normality of papillary markings on liver therapy in the Wisconsin General Hospital. A fairly common experience in this relation is intimated in Minot and Murphy's insistence [16] that the liver therapy be adequate. Starr [19] reported an instance of the recurrence of glossitis in a patient who had been relieved by liver, at a later period, on the reduction and discontinuance of this therapy. Isaacs, Sturgis and Smith [20] modified their point of view thus, "The tongue, which is frequently sore when the treatment is first begun, usually becomes normal. However, the glossitis may recur in a mild way during the remission, as the ingestion of liver does not seem to be entirely specific for this symptom." Isaacs [21] pointed out further the tendency for periodic or seasonal waves of relapsing symptoms of a mild order in patients with pernicious anemia even though the blood remission be maintained by liver therapy. Among such manifestations may be a return of the glossitis.

In a considerable series of patients with pernicious anemia on liver therapy in the Wisconsin General Hospital, the recurrence of the subjective complaint of rawness or soreness of the tongue with or without objective evidence of glossitis has served as a valuable clinical guide. As a rule, the responsibility has been traced to some intercurrent infection or advancing central nervous involvement that might reduce the efficacy of the liver dosage. Not infrequently this symptom has portended an early decline in the erythrocyte level of patients whose maintenance dosage of liver was being established. An increase in the amount of liver exhibited has controlled a majority of these cases. Occasionally patients on adequate liver therapy have been encountered in whom a glossitis has appeared without any other demonstrable change, subjective or objective. Close inquiry into their social history has revealed some psychic or physical factor of unusual stress, the control of which has relieved the discomfort. More unusual has been the

17. Starr, P.: Results of Liver Feeding in Pernicious Anemia, Am. J. M. Sc. **175**:312, 1928. Heath, E. H.: Pernicious Anemia Treated with Liver Diet and Liver Extract, J. A. M. A. **91**:928 (Sept. 29) 1928. Ungley, C. C.: The Stomach and Pernicious Anemia, Newcastle M. J. **10**:14 (Oct.) 1929.

18. Huston, John: Further Observations with the Diet Rich in Liver for the Treatment of Pernicious Anemia, Am. J. M. Sc. **174**:520 (Oct.) 1927.

19. Starr (footnote 17).

20. Isaacs, R.: Sturgis, C. C., and Smith, M.: Treatment of Pernicious Anemia, J. A. M. A. **91**:1687 (Dec. 1) 1928.

experience of Isaacs,[21] previously related; but a few instances of this order have been met.

The recent developments in the knowledge of the anemias, particularly the surpassing contributions of Castle [22] to the relationship between achylia gastrica and pernicious anemia, have given a new significance to the tongue changes of this and allied conditions. The possible causal relationship between a deficiency or lack of the intrinsic factor of Castle and the glossitis of pernicious anemia and other conditions early suggested itself; however, an isolated test of Lewis [2] would seem to indicate that an absence of the intrinsic factor does not induce lingual atrophy. In a patient with sprue studied by him, the tongue was normal; the gastric acidity and peptic activity were normal, and the blood picture was that of pernicious anemia. The gastric juice of this patient, incubated with beef and fed to a patient with true pernicious anemia, induced no remission, whereas normal gastric juice plus beef brought about a prompt response. This clinical experience is interesting and points to a dissociation between the deficiency or other factors leading to the blood picture and the lingual atrophy of pernicious anemia.

A patient with nontropical sprue, having a normal tongue and normal gastric acidity but with the history of a complete blood remission to liver therapy, presented himself for study in the Wisconsin General Hospital. Collections of the gastric juice were made daily for a month in the hope of duplicating Lewis' observations on the intrinsic factor, but no suitable subject with pernicious anemia presented himself for study. However, it was deemed advisable to attempt a further correlation of tongue changes, especially atrophy, with common laboratory findings in the hope that certain parallelisms occurring in well recognized clinical entities might afford some clue to the basic factor.

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21. Isaacs, R.: Systemic Relapses During Liver Induced Hemopoietic Remissions in Pernicious Anemia, Am. J. M. Sc. **178**:500 (Oct.) 1929.

22. Castle, W. B., and Locke, E. A.: Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia, J. Clin. Investigation **6**:2, 1929. Castle, W. B.: Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia: I. The Effect of the Administration to Patients with Pernicious Anemia of the Contents of the Human Stomach Recovered After the Ingestion of Beef Muscle, Am. J. M. Sc. **178**:748 (Dec.) 1929. Castle, W. B., and Townsend, W. C.: II. The Effect of the Administration to Patients with Pernicious Anemia of Beef Muscle After Incubation with Normal Human Gastric Juice, ibid. **178**:764 (Dec.) 1929. Castle, W. B.; Townsend, W. C., and Heath, C. W.: III. The Nature of the Reaction Between Normal Human Gastric Juice and Beef Muscle Leading to Clinical Improvement and Increased Blood Formation Similar to the Effect of Liver Feeding, ibid. **180**:305 (Sept.) 1930.

Many textbooks contain references to the relationship between the tongue and the gastric acidity. Riesman [23] noted that "in hyperchlorhydria the tongue is usually dark red, moist and clean." Wilkinson and Oliver [24] remarked glossitis with atrophy of the papillae among the clinical conditions associated with achlorhydria. Lewis [2] stated that "while a smooth tongue is most frequently seen accompanied by achlorhydria, and especially in some types of deficiency disease, the reverse does not hold."

EXPERIMENTAL DATA

Because of the apparent lack of unanimity of opinion relative to this point, the first line of attack lay in the direction of a correlation of the appearance of the tongue with the gastric acidity. When the routine aspiration failed to reveal free hydrochloric acid, an injection of histamine was given, and fractional specimens of gastric juice were withdrawn. By reason of the primary interest in the association of glossitic changes with pernicious and achlorhydric anemias, the second factor of laboratory correlation was the blood picture.

In an effort to standardize the description of the tongue, an unexpected difficulty was encountered in the prolixity of terms in the literature. A sharp limitation of such descriptive terms was deemed advisable, and for working purposes the following classification has been found quite adequate:

1. Normal tongue
2. Coated tongue (furred)
3. Scrotal tongue (furrowed, with apparently hypertrophic ridges or plaques)
   (*a*) Smooth (papillae atrophied or denuded)
   (*b*) Coated
4. Smooth tongue (papillae atrophied or denuded)
   (*a*) Complete smoothing
   (*b*) Slight general smoothing
   (*c*) Marginal smoothing (of lateral dorsum)

Practically all tongues may be grouped under these headings. Salivation and dryness, injection, ulceration and fissures were also recorded as separate modifications. It will be noted that no effort has been made to distinguish between actual atrophy and denudation in tongues designated as smooth.

In any study dependent on descriptive terms to meet a changing situation, there inevitably arises the pressing need for an objective method to preserve the record of conditions from time to time. Photography of the tongue is not entirely satisfactory, and many reproduc-

23. Riesman, D.: The Tongue, in Osler, William, and McCrae, Thomas: Modern Medicine, ed. 2, Philadelphia, Lea & Febiger, 1914, vol. 3, p. 60.

24. Wilkinson, J. F., and Oliver, T. H.: Lancet **1**:66 (Jan. 10) 1931.

tions of this nature lack the detail necessary for elucidation. The coordination of technician and subject is very difficult; hence the exposure of the tongue is frequently inadequate for descriptive purposes. Accordingly, a number of other methods were pursued with the thought of developing a technic capable of repeated application with a minimum of distortion.

The application of the ferric chloride-tannic acid method used by Elsom [25] to make foot prints was fruitless in the case of the tongue. The mixture of the tincture of ferric chloride, glycerin and water applied to the tongue invariably ran and blurred the print on paper, so that the subsequent addition of tannic acid in whatever form failed to give clearcut tongue prints. Citric acid solutions were next applied to the tongue, and the transfers to paper were brought out by heating. The detail of such prints was somewhat better, but not satisfactory. Various aniline dyes failed to meet the need. Of the several liquids tried, inks of the quickly drying type, as india, came closest to meeting the requirements.

Tracings on smoked paper, as utilized by Isaacs, Sturgis and Smith,[20] were eventually adopted as affording the best results. The materials used were smoked glazed paper cut into rectangles 3 by 5 cm., thin white cards of the same dimensions, shellac diluted 1:5 in alcohol, a shallow dish and surgical gauze or absorbent cotton. The clips and frame for drying dental x-ray films proved most convenient for handling the smoked paper rectangles. In the application of this procedure the patient was fully instructed as to the purpose and plan. All dentures were removed. The tongue was blotted carefully with gauze and its position arranged by instruction as to protrusion, flattening, etc., to effect the most regular contour. Excessive salivation occasionally necessitated packing absorbent cotton pledgets beneath the tongue. The smoked paper was then applied to the protruded tongue with just sufficient pressure from the finger tips to bring uniform contact. In removing the smoked paper, the resultant negative print was then preserved by dipping into shellac and drying. The carbon or soot adhering to the tongue suggested the possibility of a second record. By dipping the thin white card into shellac and holding it in the air just short of drying before application to the tongue, a positive record may be obtained through the soot retained on the tongue. In many instances this positive print was more delicate in detail than the original or negative print. These records were then mounted on cardboard for permanent filing. Characteristic tongue prints, positive and negative, are shown in figure 1.

25. Elsom, J. E.: Personal communication to the authors.

The physical nature of the tongue and its moisture offer certain handicaps not encountered in making finger prints; but with patience and careful adherence to the outlined technic, prints of the tongue may be taken that compare favorably in detailed accuracy with finger

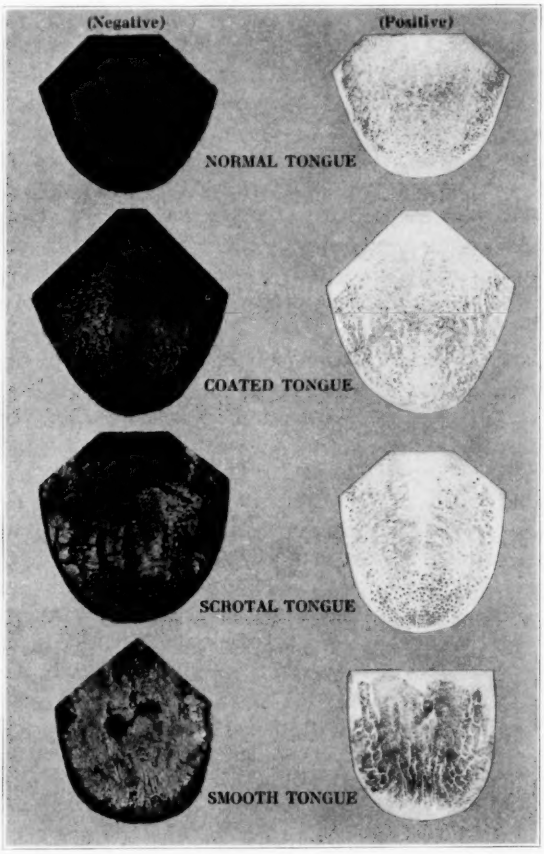

Fig. 1.—Tongue prints arranged for a comparison of the several types.

prints (fig. 2). With practice the technic proved relatively constant, and the interpretation of an isolated print usually classified the tongue as had the visual impression. The great advantage of the tongue prints lies in the fact that they constitute a permanent record, and for progress studies they should prove of inestimable value. Observations now in progress on pernicious anemia would seem to indicate that loss of the

filiform papillae is the first step toward baldness of the tongue. Later the fungiform papillae flatten and disappear. Regeneration is in the reverse order, so far as visibility is concerned.

In all, one hundred and forty subjects were included in this study. The tongue prints were taken one hundred and sixty times. Gastric aspirations after the Ewald meal were performed in all except six patients, and of the forty showing achlorhydria, fractional aspirations after histamine were made on thirty-four.

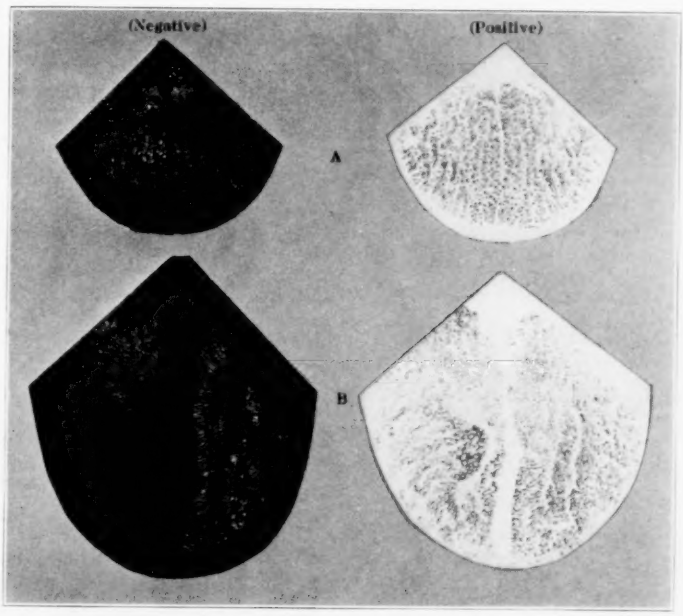

Fig. 2.—Tongue prints to demonstrate the accuracy of detail. *A*, detail of the tip of an atrophic scrotal tongue. *B*, a coated scrotal tongue, showing a scar to the left of the midline of the dorsum.

The succeeding tables have been extracted from the correlated data under the headings named.

THE TONGUE IN PERNICIOUS ANEMIA

The Correlation of the Type of the Tongue with Various Factors in Pernicious Anemia.—There were nine cases. Seven patients had some smoothing: one complete and one slight, general smoothing; four, smooth margins, and one, a smooth scrotal tongue.

Effect of Treatment: (Four patients treated, five not treated.) In the four cases in which treatment was given, two tongues were normal, one had smooth margins and one was smooth scrotal. All of the five patients not treated showed some degree of smoothing (three smooth margins, one slight general smoothing and one entirely smooth).

Effect of Involvement of the Nervous System: (Two cases normal; seven with combined degeneration). In both normal cases the patients had been treated, and both had marginal smoothing. Of the seven with combined sclerosis, five had some smoothing, one being scrotal as well, and two were normal.

Effect of Blood Changes: (Three moderately anemic; six markedly so; none normal). In cases of moderate anemia, the tongue of two patients had smooth margins and one was of the smooth scrotal type. The six cases of marked anemia included four tongues smooth in various degrees and two normal tongues.

TABLE 1.—PERNICIOUS ANEMIA

		Smoothing			Scrotal			
	Cases	Complete	Slight General	Smooth Margins	Smooth	Coated	Coated	Normal
Treated patients	4	..	..	1	1	..	..	2
Untreated patients	5	1	1	3	..	..	..	..
Normal nervous system	2	..	..	2	..	..	..	..
Combined degeneration	7	1	1	2	1	..	..	2
Moderate anemia	3	..	..	2	1	..	..	..
Marked anemia	6	1	1	2	..	..	..	2

THE TONGUE IN GASTRIC ACIDITY

The Correlation of the Type of Tongue with the Degree of Gastric Acidity.—When the gastric acidity was normal (sixty tongues), 5 per cent had slight general smoothing; 10 per cent had smooth margins; 20 per cent were scrotal; 40 per cent were coated, and 25 per cent were normal.

When there was hyperchlorhydria of any degree (30 degrees or more), there was 10.6 per cent smoothing; 23.7 per cent were scrotal, 44.7 per cent coated, and 21 per cent normal. In general, the higher the acidity, the more notable was the tendency to the scrotal type and coating of the tongue.

In cases of complete achlorhydria (thirty-nine tongues), 48.7 per cent had some degree of smoothing, and 18 per cent more were of the smooth scrotal type. Only 28.2 per cent had normal or coated tongues (7.7 per cent normal; 20.5 per cent coated). The tendency in a small series of six patients with achlorhydria not receiving histamine was not notable. In fifteen cases in which there was a small amount

of free hydrochloric acid after histamine, 46.6 per cent had some degree of smoothing, chiefly marginal; 33.3 per cent of the tongues were scrotal (13.3 per cent smooth scrotal), and 19.9 per cent were normal or coated. When these three divisions are considered together (sixty tongues), there was a general smoothing of 15 per cent, marginal of 31.6 per cent and some smoothing of 61.6 per cent (including smooth scrotal). Twenty-six per cent were scrotal, 21.6 per cent coated and only 6.6 per cent normal.

In seven cases of achlorhydric anemia, all tongues (100 per cent) were smooth in some degree (four slight general smoothing; three marginal).

TABLE 2.—GASTRIC ACIDITY

		Smoothing			Scrotal			
	Tongues	Complete	Slight General	Smooth Margins	Smooth	Coated	Coated	Normal
Normal acidity	60	..	3 5%	6 10%	5 8.3%	7 11.6%	25 40%	14 25%
Hyperchlorhydria	38	..	2 5.3%	2 5.3%	2 5.3%	7 18.4%	17 44.7%	8 21%
Achlorhydria with histamine.	39	1 2.5%	6 15.4%	12 30.8%	7 18%	2 5.1%	8 20.5%	3 7.7%
Achlorhydria without histamine	6	..	1	1	..	1	3	..
Hypochlorhydria	15	..	1	6 46.6%	2 13.3%	3 20%	2 13.3%	1 6.6%
Total hypochlorhydria and achlorhydria	60	1 1.6%	8 13.3%	19 31.6%	9	6 26%	13 21.6%	4 6.6%
Achlorhydric anemia	7	..	4 57%	3 43%	..	..	..	..

THE BLOOD AND SEX IN GASTRIC ACIDITY

The Correlation of Gastric Acidity with the Blood Picture and Sex.—Sixty per cent of the patients with achlorhydria after the administration of histamine had no anemia; 40 per cent had some degree of anemia, and 29 per cent had marked anemia. All cases with a grossly reduced gastric acidity (fifty-one) showed about the same ratio.

This figure is relatively high when compared with forty-nine cases with normal gastric acidity, of which 83.6 per cent had no anemia, and 16.3 per cent showed some degree of anemia. Only one patient (with a carcinoma) had marked anemia.

None of the fifteen patients with slight hyperchlorhydria (from 30 to 40 degrees free hydrochloric acid) had anemia. Of the eighteen patients with marked hyperchlorhydria (40 degrees or more of free hydrochloric acid), sixteen (89 per cent) had no anemia; one patient with an ulcer had marked anemia; one had slight anemia. Of a total of thirty-three cases of hyperchlorhydria, 94 per cent showed a normal blood picture.

The sex in the various acidity groups was about equally divided. Of the patients with normal acidity, 44 per cent were male and 56 per cent female. Of those with hyperchlorhydria, 42 per cent were male and 58 per cent female. Of those with achlorhydria, 56 per cent were male and 44 per cent female (including eight cases of pernicious anemia). The seven cases of achlorhydric anemia (100 per cent) were in females.

Further Analysis, Showing the Gastric Acidity and Blood Picture in Relation to the Tongue Types.—Achlorhydric anemias, all of which showed some degree of smoothing of the tongue, are not analyzed in this connection.

TABLE 3.—BLOOD PICTURE AND SEX IN GASTRIC ACIDITY

		Blood Picture			Sex	
	Cases	Normal Blood	Slight Anemia	Marked Anemia	Male	Female
Achlorhydria after histamine	34	20 60%	4 11.7%	10 29%	19 56%	15 44%
Achlorhydria with no histamine	6	5	..	1	5	1
Hypochlorhydria after histamine	11	6	4	1	3	8
Total with hypochlorhydria or achlorhydria	51	31 60%	8 15.6%	12 24.3%	27 53%	24 47%
Normal acidity	49	41 83.6%	7 14.3%	1 2%	.. 44%	.. 56%
Hyperchlorhydria; 30-40 degrees of free hydrochloric acid	15	15 100%	..	..	..	..
Hyperchlorhydria; 40 degrees and upward of free hydrochloric acid	18	16 89%	1 5.5%	1 5.5%	..	..
Total with hyperchlorhydria	33	31 94%	1 3%	1 3%	14 42%	19 57%

The Smooth Tongue: One tongue was entirely smooth; the case was achlorhydric. Of thirteen patients whose tongues showed slight general smoothing, seven had achlorhydria, one hypochlorhydria, two hyperchlorhydria and three normal acidity. Of twenty-seven patients whose tongues had smooth margins, thirteen had achlorhydria, six hypochlorhydria and two hyperchlorhydria, and six were normal.

The Scrotal Tongue: Among sixteen patients whose tongues were of the smooth scrotal type, seven had achlorhydria, two hyperchlorhydria, two hypochlorhydria and five normal acidity. Of twenty patients with tongues of the coated scrotal type, three had achlorhydria, three hypochlorhydria, seven hyperchlorhydria and seven normal acidity.

The Coated Tongue: Of the fifty-five patients with coated tongues, eleven had achlorhydria, two hypochlorhydria, seventeen hyperchlorhydria and twenty-five normal acidity.

The Normal Tongue: Of twenty-six patients with normal tongues, only three had achlorhydria, one hypochlorhydria and eight hyperchlorhydria; fourteen showed normal acidity.

The Blood Picture: Eleven of fourteen patients with general smoothing (slight and complete) were anemic (78 per cent); thirteen of twenty-seven patients with smoothed margins were anemic (48 per cent); ten of sixteen patients with smooth scrotal tongues were anemic (62 per cent); only two of twenty patients with coated scrotal tongues were anemic (10 per cent); six of fifty-five patients with coated tongues were anemic (10.9 per cent); four of twenty-six patients with normal tongues were anemic (15 per cent).

Relation of Oral Symptoms to the Type of Tongue, Blood Picture and Gastric Acidity.—Fifteen patients had the complaint of sore tongue or mouth (nontraumatic), and eleven had some degree of smoothing of the tongue (one complete; three slightly generalized; seven smooth margins). Of this group, only one had a normal tongue. Eleven of the fifteen were anemic; six markedly so. Nine patients had achlorhydria, two hypochlorhydria, two hyperchlorhydria and two normal acidity.

Three of the patients in the foregoing group complained of dysphagia (nonneurologic) and five of dry throat, and their tendencies were about the same.

The complaint of sialarrhea and the presence of salivation (six cases) had no regular relationship to the tongue picture, the gastric acidity or the blood. Two tongues had smooth margins, one was coated scrotal and three were coated. Two patients had slight anemia. Three patients had hyperchlorhydria, two achlorhydria and one hypochlorhydria.

Only one patient complained of lack of the sense of taste. He had a coated tongue, hyperchlorhydria and a normal blood count. None other was found who did not respond to the simplest test for the sense of taste.

Eighteen tongues were noted to be injected. Eleven of these were somewhat smooth (six on the margins). Three were scrotal. Only one case was normal. Six of the patients had achlorhydria; three others had hypochlorhydria; six had hyperchlorhydria, five markedly; only three had normal acidity. Eight of the eighteen patients were somewhat anemic.

THE TONGUE IN GASTRO-INTESTINAL DISEASES

The Correlation of Tongue Types and Gastric Acidity with Gastro-Intestinal Diseases.—There were twenty-two cases of peptic ulcer (confirmed by the x-rays). Of this group, only one tongue had smooth margins (4.5 per cent); 23 per cent were coated scrotal; 68

per cent were coated, and one was normal. Gastric analyses on twenty of these patients showed 40 per cent to have normal acidity; 50 per cent had hyperchlorhydria, and 10 per cent had hypochlorhydria (one patient had undergone a gastro-enterostomy).

Forty-six patients (not including those with anemia) had chronic catarrhal colitis with spastic constipation (roentgen studies after a barium enema). Four tongues (8.7 per cent) showed some smoothing, mostly marginal; six (13 per cent) were scrotal (four coated); twenty-four (52.3 per cent) were coated, and twelve (26 per cent) were normal. Gastric analyses on forty-four of the patients showed 52 per cent to have normal acidity; 30 per cent had hyperchlorhydria, and 18 per cent had achlorhydria or hypochlorhydria after the administration of histamine.

TABLE 4.—GASTRO-INTESTINAL DISEASES

		Smooth			Scrotal					Achlorhydria				
	Cases	Complete	Slight General	Smooth Margins	Smooth	Coated	Coated	Normal	Cases of Gastric Aspiration	With Histamine	Without Histamine	Hypochlorhydria	Hyperchlorhydria	Normal Acidity
Peptic ulcer.....	22	..	0	1 4.5%	..	5 22.7%	15 68%	1 4.5%	20	1 5%	..	1 5%	10 50%	8 40%
Chronic colitis.....	46	..	1 8.7%	3	2 13%	4	24 52%	12 26%	44	5	1 18%	2	13 30%	23 52%
Chronic cholecystitis	33	..	1 12%	3	1 18%	5	13 40%	10 30%	32	3	2 32%	5	8 25%	14 43%
Carcinoma of stomach	5	..	1 20%	1 20%	..	..	3 60%	..	5	2	1	1	0	1
Neurosis.....	9	..	..	0	1	..	6	2	9	1	..	..	3 33%	5 55%

Thirty-three cases were diagnosed as chronic cholecystitis (confirmed by the x-rays after the intravenous injection of dye). Four tongues (12 per cent) showed smoothing, mostly marginal; six tongues (18 per cent) were scrotal (five coated); thirteen tongues (40 per cent) were coated, while ten tongues (30 per cent) were normal. Gastric analyses on thirty-two of the patients showed 43 per cent to have normal acidity; 25 per cent had hyperchlorhydria, and 32 per cent had a tendency to achlorhydria.

Five cases of carcinoma of the stomach were studied. Partial smoothing was noted in two and coating in three. The patients all had very low acidity, three being achlorhydric.

Nine patients were diagnosed as having a neurosis. None of them had smooth tongues; six tongues were coated, one was scrotal and two were normal. (Five of the nine patients had normal acidity, three had hyperchlorhydria and one was achlorhydric.)

MISCELLANEOUS DISEASES WITH CORRELATED DATA ON THE TONGUE TYPE AND GASTRIC ACIDITY

Two patients with the hyperacidity syndrome had scrotal tongues.

Five diabetic patients had coated scrotal tongues; all had normal or slightly increased gastric acidity. The beefy red tongue of diabetic ketosis is familiar to all, however.

Three cases of ulcerative colitis included two normal and one coated tongue. Two patients had normal acidity; one had hyperchlorhydria.

DEDUCTIONS

The observations incorporated in this report were made on an unselected group of subjects within a limited range of clinical conditions. Any deductions drawn therefrom must bear these limitations in mind. Obviously, broad generalization or attempted application to unrelated diseases would be unwarranted.

1. The series of cases of pernicious anemia is small. All of the patients not treated showed papillary atrophy of some degree. Neither the changes in the spinal cord nor the grade of anemia had any independent influence on the gross appearance of the tongue.

2. In this study patients with hyperchlorhydria rarely had smooth tongues, but often had scrotal or coated tongues. When the gastric acidity was normal, there was occasional smoothing, with a somewhat less tendency toward coating and a greater tendency toward normal tongues. When there was achlorhydria or a tendency toward it, smoothing of the tongue, or the clean scrotal type, was much more common. The corollaries to these tendencies seemed equally true.

3. In this series the patients with achlorhydria were often anemic; when the free hydrochloric acid was normal, this tendency was less pronounced, and when hyperchlorhydria was present, the occurrence of anemia was rare. When achlorhydria and anemia were combined in true achlorhydric anemia, the tongues were all somewhat smooth; the patients were all females.

4. From these observations the incidence of anemia would appear to bear a relationship to the degree of smoothing of the tongue. Normal and coated tongues were not commonly associated with anemia.

5. The subjective complaints of sore tongue and mouth, dysphagia and dry throat occurred frequently in association with the objective findings of a smooth tongue, anemia and achlorhydria. Injection of the tongue also appeared commonly with smoothing and anemia, but with either a marked hypo-acidity or a hyperacidity.

6. In organic nonmalignant disease of the gastro-intestinal tract, there was a tendency to the coated and the scrotal types of tongue.

This was more marked when hyperchlorhydria coexisted and was apparently proportional to the degree of hyperacidity. Peptic ulcer showed the most marked tendency in this direction, with chronic colitis and chronic cholecystitis following in order. Functional conditions were rarely attended by smoothing of the tongue, and had a tendency to occur in the presence of normal gastric acidity.

7. Observations on two cases of achlorhydric anemia over periods of three and four months, respectively, did not show appreciable changes in the tongue even though the hemoglobin had returned to normal. A patient with pernicious anemia inadequately treated in terms of blood response to liver therapy gave an entirely normal tongue print, demonstrating an early return of papillae to the affected member. The dissociation of the causative factors in the anemia and the glossitis of these two conditions would seem thereby to be established.

Book Reviews

A Manual of Clinical Laboratory Methods. By C. L. Cummer. Third revised edition. Cloth. Price, $6.75. Pp. 585, with 173 engravings and 12 plates. Philadelphia: Lea & Febiger, 1931.

This third revised edition of Cummer's manual deserves to meet the kindly reception given his original and second publications. Those were considered excellent guides for clinical laboratory procedures and texts in courses of laboratory technic. The new edition, with its expanded section on the Kahn reaction, the new section on Kline's reaction, as well as addenda or revisions to various other chapters, contains important additions. The twelve chapters deal with the usual technic for examining the blood, the morphology of its formed elements and their variations; the changes of the blood with the anemias and leukemias; the animal parasites and bacteriology of the blood; the immune reactions; the examinations of the chemical constituents of the blood; the procedures for the routine examination of the urine and renal function; the examination of the gastric and duodenal contents; the examination of feces, the changes in certain diseases and animal parasitism; the technic for sputum examination; the procedures in the examination of the various fluids of the body, diseases of the skin, etc.; basal metabolism, and bacteriologic technic. An appendix includes directions for preparing solutions and stains.

Female Sex Hormonology. By William P. Graves, A.B., M.D., F.A.C.S., Professor of Gynecology at Harvard Medical School; Surgeon-in-Chief to the Free Hospital for Women and to the Parkway Hospital, Brookline, Mass. Cloth. Price, $3.50. Pp. 131, with illustrations. Philadelphia: W. B. Saunders Company, 1931.

This is a reliable and useful monograph, the kind one has a right to expect from a representative of the Harvard Medical School. The treatment is selective rather than exhaustive, the author having endeavored to outline those steps in the development of this important subject that have led to definite scientific and clinical results. The final chapter is on organotherapy; the other remaining ten chapters deal briefly with all the phases of sex life of the mammalian female, particularly the human species. The author, modestly, notes in the preface that the book is primarily designed for the student who has little previous knowledge of the subject. But the character of the monograph is such that it can and should be read thoroughly by every general practitioner of medicine, for the reason that the confusion in this field is still great, particularly on the side of organotherapy. The reports of real advances are scattered in many journals and in many tongues so that it is difficult for a busy practitioner to keep up with them. The author has rendered a real service to the busy physician in preparing this essentially critical, objective and sound digest. The only criticism that the reviewer might direct against the author is the inclusion on page 103 of some of the organotherapy preparations available or recommended for the treatment of sex disorders in women, preparations nonstandardized and in such forms that they are to be or must be taken by mouth. Such preparations no longer have any place on a reliable list of female sex hormones for clinical use.

Modern Medical Treatment. Volumes I and II. By E. Bellingham Smith, M.D., F.R.C.P., London; Physician and Lecturer on Medicine, St. George's Hospital, and Anthony Feiling, M.D., Cambridge, F.R.C.P., London; Physician and Lecturer on Medicine and Dean of the Medical School, St. George's Hospital. With an introduction by Sir Humphrey Rolleston, Bart, G.C.V.O., K.C.B. Price, $12 per set. Pp. 622 and 626. New York: William Wood & Company, 1931.

These volumes, covering the whole field of internal medicine, are of chief interest because they reflect present-day treatment as employed by the English school. In them is to be found an amazing combination of what has been accepted as of empiric value, as well as recent methods based on scientific clinical research. Many procedures are recommended which are widely different from those in common use in America. The work is chiefly of value to Americans because of suggestions of value which have been handed down by our forefathers, but which are rarely used by the present generation. The use of drugs in the treatment for disease is given prominence. Many prescriptions, in instances suggesting polypharmacy, are recommended throughout the work, and in this respect it indicates a type of therapeutics in marked variance to the trend in America. Some chapters, notably the chapter on the treatment for goiter, can hardly be accepted by those in America who have given this subject special study. Dietetics receives considerable attention; detailed dietetic formulas are given for many diseases. The work carries an introduction by Sir Humphrey Rolleston, which is of decided interest. An extensive index is printed in duplicate and is to be found at the end of each volume.

Die Schleimhäute bei der vasoneurotischen Diathese. By Ludolph Fischer, Dr. med., Privatdozent für innere Medizin an der Universität Tübingen. Mit einem Nachwort von Ottfried Müller, Ordentl. Professor, Dr. med., Vorstand der medizinischen Klinik Tübingen. Paper. Price, 10 marks. Pp. 68, with 85 illustrations and 1 plate. Stuttgart: Ferdinand Enke, 1931.

This study of accessible mucous membrane with special reference to the arrangement of the capillaries was stimulated by Ottfried Müller, who is one of the formulators of the "vasoneurotic diathesis." The mucous membranes of the lips, gums, tongue, rectum, conjunctiva and cervix were observed through a binocular magnifying glass, and careful drawings were made of the capillaries. In a general way, the capillary morphology is the same in the skin and the various mucous membranes. Compared with normal persons, those with the "vasoneurotic diathesis" were found to have more irregular capillary formations throughout, which is considered strong evidence for the constitutional and fundamental nature of this diathesis. That this diathesis predisposes to gastric ulcer has been shown by study of excised pieces of gastric mucosa from patients with ulcers. It is claimed that people with characteristic "vasoneurotic" skin capillaries may be assumed to have similar conditions of the mucous membranes and that they are predisposed to such diseases as gastric ulcer, asthenia and hypertension.

A Manual of Tuberculosis for Nurses. By E. Ashworth Underwood, M.D., Ph.D. Cloth. Price, $2.50. Pp. 272, with illustrations. New York: William Wood & Company, 1931.

Because of the great advances during the past decade in the care and treatment of patients with tuberculosis, the nursing of tuberculous patients has come to be a special field demanding specialized instruction and practice.

This manual provides in a clear and convenient form the information required by nurses who are engaged in this work in the sanitorium, hospital or home, and should serve as an excellent text for ready reference and also for use in preparing for examinations to qualify for this type of nursing.

It is printed in large, clear type on good paper. The illustrations are well chosen. There are a glossary of technical terms and an index.